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# THE AMERICAN JOURNAL OF PATHOLOGY

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## PURE GRANULOMATOUS NOCARDIOSIS: A NEW FUNGUS DISEASE DISTINGUISHED BY INTRACELLULAR PARASITISM

A DESCRIPTION OF A NEW DISEASE IN MAN DUE TO A HITHERTO UNDE-  
SCRIBED ORGANISM, *NOCARDIA INTRACELLULARIS*, N. SP., INCLUDING A  
STUDY OF THE BIOLOGIC AND PATHOGENIC PROPERTIES OF THIS SPECIES\*

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Phagocytosis by reticulo-endothelial cells, together with proliferation of these cells, resulting from the presence of an offending agent, has been regarded generally as the fundamental basis of granulomatous inflammation. The majority of these reactions quickly become complicated by necrosis, fibrosis, alteration in structure of the macrophage, and appearance of other cellular elements such as lymphocytes and plasma cells. In human disease one rarely encounters a granulomatous reaction consisting entirely of phagocytosis and proliferation of macrophages. This is most nearly approached in the lesions of typhoid fever, histoplasmosis, and early stages of lymphogranuloma inguinale, but even here complicating features are to be found.

Opportunity was afforded recently to study a human reaction which assumed the form of phagocytosis with reticulo-endothelial cell proliferation and minimal necrosis. The agent which induced this reaction is an acid-fast organism which may be isolated easily in culture.

### REPORT OF CASE

The patient was a white girl, 34 months old, whose illness began with anorexia, nausea, vomiting, and progressive weight loss about 4½ months before her death. Shortly after the onset a mass, thought to be a lymphosarcoma, was noted in the abdomen. The urine was reported as negative; hemoglobin, 8.4 gm. (52 per cent); red blood cells, 3,100,000; white blood cells, 20,150 (polymorphonuclear leukocytes, 33 per cent; eosinophils, 3 per cent; lymphocytes, 62 per cent; monocytes, 2 per cent). A transfusion of 225 cc. of type A blood and five treatments of deep x-ray therapy (dosage not known) were administered. Because of unfavorable reaction,

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radiotherapy was discontinued. She then was admitted to Duke Hospital for an exploratory operation.

*Physical Examination.* The child was emaciated; her skin was pale and dry. The lymph nodes of the axillary, cervical, and inguinal groups were enlarged. The chest was clear to percussion and auscultation; the heart showed no abnormality. Pulse was 120; blood pressure, 88/50 mm. Hg. In the abdomen, prominent in the upper quadrants, was a large, irregular mass. There was diminished muscular tone, especially of the extremities and abdomen.

*Course.* Shortly after admission an enlarged inguinal node was removed for study. The node showed complete alteration of its architecture with proliferation of the macrophages (Fig. 1). The cytoplasm of these macrophages appeared foamy and granular. Inasmuch as they resembled Gaucher's cells, a lipodystrophy was considered. However, by the use of Ziehl-Neelsen stain it was demonstrated that these cells contained massive numbers of an acid-fast bacilliform organism. A second biopsy showed a lesion of essentially the same type, and the organism was isolated in pure culture. Before the first biopsy was completed, the patient had received five x-ray treatments of 50 r., but upon the discovery of acid-fast organisms radiotherapy was discontinued. She then was treated by supportive therapy with intravenous supplemental proteins (amigen) and by chemotherapy, specifically, sodium p,p'-diaminodiphenylsulfone-N,N' didextrose sulfonate (promin) and penicillin. The entire hospital course was afebrile except for two minor elevations of temperature to 38.5°C. and 38.8°C., respectively. The blood counts fluctuated: Hemoglobin, 8.1 gm.; red blood cells, 4.2 millions; white blood cells, between 7,500 and 19,360, with a differential formula of 26 per cent polymorphonuclear leukocytes, 38 per cent staff cells, 19 per cent juveniles, 4.5 per cent lymphocytes, and 12.5 per cent monocytes. Serologic tests for syphilis were negative. Total plasma proteins were 3.6 gm. per 100 cc.; albumin, 1.3 gm. per 100 cc.; globulin, 2.3 gm. per 100 cc.; albumin/globulin ratio, 0.56; cholesterol, 100 to 110. About 2 months before death the stools contained 30.49 per cent fat, with no parasites or ova; but many acid-fast organisms were found by both smears and culture. Other laboratory data were noncontributory.

On proctoscopic examination irregular ulcers were observed. A biopsy of one of these ulcers showed proliferation of macrophages containing many acid-fast organisms. Examinations of nasal smears for acid-fast organisms were negative. Intradermal tests with old tuberculin and avian tuberculin gave negative results, although the patient's serum was said to have agglutinated a suspension of her own organisms in a dilution of 1:640. The patient grew progressively weaker despite supportive measures, and died on her 78th hospital day, 4½ months after the onset of her illness.

#### AUTOPSY FINDINGS

##### *Gross Examination*

The body was emaciated, with accentuation of bony prominences. The *abdomen* was conspicuously distended. When the *abdominal cavity* was opened, approximately 500 cc. of chylous fluid escaped. Similar fluid was found in lesser quantity (150 cc.) in both *pleural cavities*. The *lacteal vessels* on the surface of the intestines were congested. The abdominal organs were displaced by a large, space-consuming mass in the mesentery and retroperitoneal regions (Fig. 2). This mass consisted of greatly enlarged and matted *lymph nodes*, which

on section were brilliant yellow. In the central portion of this mass were scattered, irregular zones of necrosis. The *pancreas*, *kidneys*, and *adrenals* were imbedded in the mass but not infiltrated by it, and were not otherwise abnormal. The *small intestine* showed no lesions, but ulcers occurred throughout the length of the *colon* (Fig. 3). These ulcers were eroded and indurated, with overhanging edges. Their bases were covered by blood-stained, greenish, necrotic material. The *lymph nodes* near the intestine were enlarged and bright yellow. The *spleen* was only slightly enlarged, and scattered throughout were yellowish areas which were interpreted grossly as malpighian corpuscles. The *liver* showed a prominent architecture with no focal lesions. The *mediastinal lymph nodes* were slightly enlarged. The *heart* and *lungs* were without gross lesions. The *brain* appeared normal. The *bone marrow* of the sternum, vertebra, and femur was pale, but gross lesions were not recognized.

### *Microscopic Examination*

The architecture of the *spleen* was greatly distorted by an abundant proliferation of large, pale, foamy-appearing macrophages to the extent that lymphoid remnants appeared only as focal collections of cells (Fig. 4). The malpighian corpuscles were replaced by epithelioid cells, concentrically arranged about the central artery (Fig. 8). These structures were delineated by what appeared to be collapsed sinusoids containing small quantities of blood. Lymphocytes were virtually absent, although a few scattered plasma cells could be found. There was no conglomeration of these structures, despite their juxtaposition. The epithelioid cells contained numerous intracellular acid-fast organisms (Fig. 10), simulating the parasitism found in leprosy and Johne's disease. There were a few isolated organisms of varying lengths in the interstices. Branching forms were not observed. Groups of multinucleated giant cells, usually in clusters of a dozen or more, occurred throughout the spleen. The nuclei were in a peripheral position, and, for the most part, were small and hyperchromatic; only a few of the pale, vesicular type were found. In the central zone of the cytoplasm there was a clear, spherical, homogeneous area (Figs. 9 and 11) which proved to be devoid of organisms, but the foamy, granular cytoplasm around it contained acid-fast organisms, usually radially arranged. Foot's silver preparations failed to demonstrate reticulum, and even with Masson's trichrome stain little fibrosis was recognized.

The architectural pattern of the *lymph nodes* was as distorted as that of the spleen (Fig. 5), and there were similar groups of large giant cells



(Figs. 8 and 11). The epithelioid pattern found in the spleen was nowhere apparent in the nodes. In some zones the multinucleated giant cells were so numerous as to form broad sheets. Isolated areas of necrosis were found in the retro-aortic and mesenteric lymph nodes. Only a few of these areas were large. Elsewhere necrosis was not a feature of the lesion. Several recanalized thrombi in fairly large arteries were noted.

The floor of the *ulcers in the colon* consisted of fibrous scar tissue, with intermingled groups of macrophages containing very numerous acid-fast organisms. There were remarkably few leukocytes and plasma cells, commonly seen in intestinal ulcers. Groups of macrophages with phagocytized organisms were noted also in nonulcerated areas, interpreted as replacement of lymphoid follicles by reticulo-endothelial cells. They frequently interrupted, by infiltration, the muscularis mucosae. The ulcers were thought to arise by surface erosion of the masses of proliferating macrophages.

Scattered tubercle-like nodules of epithelioid cells occurred in the *liver* (Fig. 6). The epithelioid cells contained many acid-fast organisms (Fig. 7). Many Kupffer cells throughout the liver showed phagocytosis of the organism, but there was no apparent damage to liver cells. In routine preparations of the *lung* no lesions were found. However, by acid-fast technics, organisms were demonstrated in the submucosal lymphoid follicles of the bronchi and in perivascular macrophages. Here again, massive intracellular parasitism was apparent.

In the mucosa of the *appendix* the lymphoid element was somewhat proliferative, and here also were many intracellular acid-fast organisms; but no tuberculoid arrangement was seen. The tissue about the *kidneys*, *pancreas*, and *adrenals* contained foci of epithelioid proliferation and there was intracellular parasitism. However, lesions were not found within the parenchyma save for scattered calcium deposits in the medullary tubules of the kidney.

In sections of the *myocardium*, calcific subendocardial deposits were found in the vicinity of the mitral valve, and there was a mild vacuolization of myocardial fibers, but no granuloma.

Microscopic studies of the *bladder*, *uterus*, *ovary*, *diaphragm*, *thyroid*, *pituitary body*, and *brain* were noncontributory. These organs appeared normal; no aggregates of organisms were found.

*Anatomic Diagnoses.* A peculiar form of granuloma characterized by intracellular parasitism and proliferation of macrophages producing complete replacement of lymphoid tissue of mesenteric, retroperitoneal, mediastinal, and left subclavian lymph nodes, and spleen; partial re-

placement of bone marrow and lymphoid tissue of gastro-intestinal tract; and microscopic proliferation of macrophages with intracellular organisms in liver and lungs; granulomatous ulceration of colon; congestion of lacteal lymphatics, chylous ascites (500 cc.), bilateral pleural effusion (150 cc.); calcium deposition in subendocardial tissue and renal tubules; and emaciation.

#### INTERPRETATION

The outstanding feature of the disease in this case was the peculiar host-parasite relationship. The sole local reaction appeared to be a stimulation of phagocytic activity of the macrophages of the reticulo-endothelial system with proliferation of these phagocytes. As a consequence, the predominant lesions occurred in the spleen, lymph nodes, and bone marrow. This feature was emphasized further by the fact that in the small lymphoid collections of the bronchi, in the liver, and around the vessels in the pancreas was found marked phagocytosis of these organisms by large macrophages. This process could not be appreciated in ordinary preparations and was recognized only when the tissue was stained by the acid-fast technic.

This host-parasite relationship was characterized further by the predominantly intracellular position of the parasite. However, in a few areas, particularly in the lymph nodes and the spleen, extracellular organisms were noted; but with the impressively dominant intracellular parasitism organisms in the interstices were interpreted as having "spilled over" from the macrophages. The organisms appeared to be in equilibrium with the cells and thus were capable of growth and multiplication. The formation of giant cells, with peripherally placed nuclei and a clear central zone, was interpreted as support for the concept of Doan, Sabin, and Forkner<sup>1</sup> regarding the formation of giant cells of the Langhans' type by amitotic division of macrophages. By supravital staining these authors have shown that the peripheral disposition of the nuclei in Langhans' giant cells is due to the centrally placed "rosettes of fine vacuoles," which in the living state are stainable with neutral red. It seems likely, therefore, that the clear central zones seen in many of the giant cells in the lymph nodes and in the spleen of this case were due to the presence of such "rosettes."

This host-parasite relationship seemed to be characterized further by the fact that no product of metabolism of the organism was toxic to its host. Such a point of view seems substantiated by the fact that throughout her illness the patient did not exhibit a consistent febrile response. In addition, there was no leukotaxis, in that none of the

lesions showed significant numbers of polymorphonuclear leukocytes or other elements of suppurative inflammation. In this connection it is noteworthy that early in her illness there was an absolute lymphocytosis. However, following x-ray therapy and progress of the disease a degenerative shift of the hemogram occurred. Necrosis was not a conspicuous feature. There were zones of necrosis in the large masses of lymph nodes of the mesenteric and retroperitoneal groups, but the more discrete nodes and the spleen did not show this feature. It may be pointed out that the large mass in the abdomen had received a total dosage of approximately 500 r. of deep x-ray therapy. In addition, two large vessels with old recanalized thrombi were found in these nodes at autopsy.

Intracellular parasitism by an acid-fast organism, as presented here, finds its analogue in human leprosy, Johne's disease in cattle, and rat leprosy. However, in human leprosy there are elements in the process of inflammation other than those which characterize the disease under consideration, and the distribution of lesions is different. Furthermore, the organism of leprosy is recovered with extreme difficulty, if it can be cultured at all. Rat leprosy of the glandular type presents a lesion which is strikingly similar to that found in our case. On comparing sections of rat leprosy obtained through the courtesy of Dr. R. D. Lillie of the National Institute of Health, we have found that, while there is extensive phagocytosis and macrophagic proliferation, the lymph node structure does not show the widespread alteration that is the rule in our case. The organism of rat leprosy is more difficult to stain and has greater variation in structure than the organism in our case. Moreover, the organism of rat leprosy has not been successfully cultured by ordinary methods.<sup>2</sup>

In Johne's disease there are reacting elements other than the reticulo-endothelial cells. The lesions are distributed chiefly throughout the gastro-intestinal tract, and the organism possesses cultural and morphologic characteristics which differentiate it sharply from that responsible for the disease in our case.

A consideration of the mechanism of death in this patient suggests a number of interesting possibilities. The patient obviously was suffering from severe inanition. While it is evident that absorption of nourishment from the gastro-intestinal tract was impaired, it does not seem likely that this factor alone could account for the severe metabolic disturbance. Moreover, the analogy of the situation to that of advanced malignant disease is sharply apparent. Perhaps the same undetermined factors which produce death in certain cases of cancer were operative

in this case. This view finds some support in the extraordinary tumor-like proliferative activity of the reticulo-endothelial cells which constituted a typical feature.

## NATURE OF THE ETIOLOGIC AGENT

### *Isolation*

Isolates of this organism were obtained first from a retroperitoneal lymph node removed for biopsy, later by culture of stools, and finally from the spleen and a lymph node at autopsy. Small acid-fast organisms were seen in smears made from the ileum, jejunum, colon, spleen, abdominal lymph nodes, urine, and ascitic fluid. They did not appear in direct smears from the heart's blood, lungs, liver, kidney, and spinal fluid, although they were subsequently stained in sections of liver and lungs. Post-mortem cultures were freed from contaminants by digestion with normal sodium hydroxide for 30 minutes. Observations were made on isolates obtained both before and after the death of the patient.

### *Cultivation*

*Gross Morphologic Features on Solid Media.* On glycerin egg medium, growth appeared in 24 to 28 hours as smooth, minute, shiny, pale yellow colonies which rapidly increased in size and pigmentation. After 3 to 7 days the colonies became confluent, definitely elevated, shiny, pale yellow to yellowish orange, and butyrous (Fig. 12). A sweet, yeast-like odor was produced irrespective of the substrate used. Aerial mycelium did not appear at any stage of cultivation, and there was no discoloration of the media. Growth on plated media, while not unlike that on slants, was somewhat slower. On Sabouraud's agar, growth was visible in 36 to 48 hours, and resembled the young colonies produced on glycerin egg slants. As growth increased, however, the color became gray to grayish yellow. With ageing the colonies assumed the characteristic deeper yellow to yellowish orange. The growth eventually became more elevated than on glycerin egg medium, and vermiform (Fig. 13). On plates of sheep's blood-agar, growth was slow, and a slight, irregular hemolysis was produced. Czapek's agar plates or slants with carbohydrate did not support abundant or characteristic growth, although subcultures grew rapidly and well when transferred from this medium to glycerin egg substrate. On Bordet-Gengou agar, colonial development was similar to that obtained on glycerin egg and Sabouraud's media. On Löffler's serum slants, pigmentation of the colonies was increased, so that a deep yellow-orange color was produced. Growth on the Löffler slants was peculiar in that it apparently terminated after

3 to 4 days. Subcultures from this to other media were normal in colony characteristics.

*Growth in Liquid Media.* Beef extract broth plus 5 per cent glycerin favorably supported growth. In 3 to 7 days a web-like mucoid clot was formed, primarily in the butt of the tube; however, there might be some extension of the growth over the dependent side of the tube. The broth remained clear and there was no pellicle. Prolonged incubation (30 to 40 days) resulted in a slight, increasing turbidity, the degree of which depended upon the amount of inoculum used. The broth was not discolored. In beef extract broth without glycerin and in beef infusion broth without glycerin there was no visible growth after 6 to 7 days. Beef extract broth containing 5 per cent glycerin and 0.5 per cent phenol supported growth which resembled the cultures in glycerinated beef extract broth. Transfers from phenolized broth grew rapidly and well, with no change in gross cultural characteristics. The growth in Sabouraud's broth was similar to that in beef extract broth with glycerin. Proskauer-Beck broth containing asparagin and Dubos' (Tween) broth supported growth, but not as well as did beef broth containing glycerin or Sabouraud's broth.

*Viability, and Oxygen and Temperature Requirements.* Cultures remained viable for months on slants of glycerin egg medium, and successful transplants were obtained from cultures 1 year old. A culture, with or without other organisms present, resisted digestion by N sodium hydroxide for a period of 30 minutes. The organism was aerobic, but grew in a candle jar containing approximately 10 per cent CO<sub>2</sub>. There was no growth in the Brewer anaerobe jar and the organism died within 14 days. The optimum temperature for growth was 37.5°C., but there was good growth at room temperatures. No growth occurred at 40°C., and the organism was killed at 60°C. in 10 minutes.

### *Biochemical Reactions*

In fermentation broth to which 1 per cent xylose or 1 per cent arabinose had been added, acid without gas was produced in 5 to 10 days. No fermentation occurred in broths containing 1 per cent of lactose, dextrose, sucrose, mannite, tréhalose, or sorbitol. In litmus milk there was a slight acidity within 20 to 30 days. Peptonization did not occur and growth seemed to be more abundant in the lower portion of the tube. After incubation for 35 days in gelatin, there was neither visible growth nor liquefaction. Indole was not formed, nor was tyrosinase produced, since there was no brownish discoloration of the peptone broth. Nitrates were reduced to nitrites. Cellulose was not decomposed in Dubos' cellulose-nitrate broth, although this medium supported a

slight growth. In potato-dextrose-starch agar little or no growth occurred, and there was no diastasis of the starch.

To determine the utilization of paraffin as the sole source of carbon, a glass rod coated with sterile paraffin was suspended in a flask of carbohydrate-free Czapek's broth. Previous to the insertion of the paraffin-coated rod a 0.2 cc. saline suspension of the organism had been added to the medium. After 7 days' incubation at 37.5°C., the paraffin-covered rod was coated with a dust-like, yellow film. The rod was streaked across the surface of glycerin-egg medium slants, and typical growth of the organism occurred in 4 to 7 days. A stain of a smear from the rod showed many acid-fast organisms.

### *Microscopic Morphology and Staining Reactions*

The organism was pleomorphic, Gram-positive, acid-fast, non-spore-forming, and when undisturbed exhibited true lateral branching. In smears of tissues and exudates from the patient and from experimental animals intracellular bacillary forms predominated, but some free bacillary and a few coccobacillary forms were present. Some of the bacillary forms contained large pleomorphic granules, and all free organisms tended to assume a diphtheroid-like arrangement. There were no ray formations in exudate. In all preparations the organism was 0.2 to 0.45  $\mu$  in diameter. The longer elements contained small spherical to ovoid bodies at regular intervals throughout their lengths. These ovoid structures were extremely acid-resistant and Gram-positive. In addition, large pleomorphic structures were found, at times terminally and sometimes irregularly placed throughout the organisms. These were wider than the remainder of the organism. They were not acid-fast, but took the counterstain intensely and were Gram-positive. It may be pointed out that in fat-enriched and glycerinated media the number of these bodies was increased.

On Sabouraud's agar in Van Tieghem cell mounts the organism consistently produced branching filaments in 5 to 8 days. These branches formed a densely matted mycelium in the central portion of the mount. Delicate, tangled, branching hyphae, 0.2 to 0.45  $\mu$  in width, were formed at the periphery of the growth (Fig. 16). There was no indication of dichotomous branching, chlamydospores, or chains of spores such as may be seen in those aerobic actinomycetes which produce an aerial mycelium. In cultures other than the Van Tieghem single cell mounts, branching was inconspicuous when examined in smears made by loop transfers (Fig. 15). However, in litmus milk long branching forms were prevalent, and large tangled masses were commonly observed (Figs. 17 and 18). Because of the consistent branching and

the growth on Sabouraud's medium at room temperature, the organism is regarded as a fungus. The small, round to ovoid, regularly placed bodies, therefore, may be interpreted as arthrospores; and the irregularly spaced, pleomorphic, non-acid-fast structures may be termed granules. Therefore, the organism appears structurally as a series of arthrospores bound together by a thin membranous sheath.

Throughout the study of this organism its consistent and extraordinary resistance to decolorization with acid or acid-alcohol was an impressive feature. In smears made from exudate and from freshly isolated cultures the organism was partially decolorized by 25 per cent hydrochloric acid-alcohol\* in 1 hour. However, preparations made from litmus milk cultures remained completely acid-fast in 25 per cent hydrochloric acid-alcohol for over 60 minutes. On the other hand, the acid resistance was so diminished by culturing on Sabouraud's medium that the organism was totally decolorized by 2 per cent hydrochloric acid-alcohol in 3 minutes. Acid-fastness was influenced in part by the hydrogen ion concentration of the medium. Increase in either alkalinity or acidity of the medium resulted in a correlated diminution of acid-fastness. The greatest resistance to decolorization was obtained by cultivation at a pH range of 7.2 to 7.6. The age of the culture, however, had no influence, for a 16-months-old culture on glycerin egg medium retained its acid-fastness to a degree comparable to that of fresh cultures.

Regardless of the medium used, the relative acid-fastness of the component parts of the organism was constant. The arthrospores were the most vividly stained by the fuchsin; in contrast the rest of the hypha, although clearly acid-fast, was pale. The granules were never acid-fast.

#### *Analysis of Characteristics*

The outstanding features of this organism are its high degree of acid-fastness, its consistent branching on media permitting direct observation, and its resistance to sodium hydroxide digestion. The acid-fastness rivals that found in the mycobacteria and exceeds that usually encountered in the nocardia. However, the acid-fastness of species of *Nocardia* varies considerably; a few strains have been described as "strongly acid-fast"<sup>3</sup> and "as resistant to acids as the tubercle bacillus."<sup>4</sup> The constant branching of the organism under investigation and its fragility as observed when transferred by loop to smears are suggestive of the aerobic strains of actinomycetes for which Waksman and Henrici<sup>5</sup> employed the generic name *Nocardia*. It is well known that branching forms of mycobacteria exist. However, they are found

\* Prepared by using 25 cc. of C.P. HCl plus 75 cc. of 95 per cent alcohol.

only rarely and under unusual conditions, as in smears from old cultures and sometimes in exudates.<sup>6</sup> This feature of mycobacteria is not consistent even in the same strain. The resistance of our organism to sodium hydroxide digestion is difficult to evaluate as a criterion of classification. This procedure is generally employed to free exudates of organisms other than *Mycobacterium tuberculosis*. However, it may be that the factors responsible for resistance to acid decolorization on the part of an organism may also protect it against sodium hydroxide digestion.

### *Comparison with Other Organisms*

*Mycobacterium tuberculosis*. As indicated above, the organism here described has a number of morphologic features in common with *M. tuberculosis*. The similarity of fragments of the hyphae to tubercle bacilli is striking, especially when the two are compared in photographs made with the electron microscope. However, the resemblance of our organism to *M. tuberculosis* goes no further than this. The consistency of its branching, its ability to grow on a multitude of media at extremely variable temperatures and hydrogen-ion concentrations, its ability to utilize paraffin as its sole source of carbon, its unique relationship to the cell of its human host, and its failure to kill experimental animals differentiate it sharply from any known form of virulent *M. tuberculosis*.

*Other Acid-Fast Bacteria*. Intracellular parasitism and acid-fastness, both important characteristics of our organism, are also characteristic of *M. leprae*. Beyond this, however, resemblance between the two completely disappears. The ease with which growth of our organism is obtained would seem to eliminate any possibility of confusing it with *M. leprae* in view of the well known difficulty and special technics required in culturing the leprosy bacillus.<sup>7-9</sup> For example, the organism isolated by Clegg<sup>8</sup> from a case of leprosy is only weakly acid-fast and extremely fastidious in its growth requirements on primary isolation, features which distinguish it sharply from our organism. The organism from Duval's<sup>9</sup> case of leprosy, unlike our organism, required 1 to 7 months on highly enriched media for primary isolation, and the colonies produced were glistening and white. A number of acid-fast bacilli have been isolated from cases of leprosy by other workers, but absolute proof that these organisms are causative of this disease is lacking.<sup>10</sup> From the studies of our own organism in its relation to the disease in the host in which it is found, and in relation to experimentally produced disease in a variety of animals, as described in a subsequent section, there is no question of its etiologic specificity.

Johne's disease of cattle and rat leprosy show intracellular parasitism by acid-fast organisms comparable to that in our case. The organism



of rat leprosy, like that of human leprosy, has not been cultured by ordinary methods,<sup>2</sup> and the organism of John's disease differs from our organism in the following ways: John's bacillus requires for its growth the addition of extracts of other acid-fast organisms to the primary isolation medium; it requires 4 weeks for growth to become apparent; dull yellowish white, striated colonies are produced; maintenance requires enriched media, and branching has not been observed.<sup>11</sup>

The remaining acid-fast organisms, considered saprophytic mycobacteria, offer problems of comparison with our organism which cannot be solved adequately. For example, statements regarding their ability to branch on Van Tieghem cell mounts are not included in their descriptions. Through the courtesy of Dr. William A. Hagan of Cornell University we have been able to compare our organism with some of these acid-fast bacteria, including one strain each of *M. phlei*, *M. ranac*, and *M. leprae* (the Clegg II bacillus), and five acid-fast soil saprophytes. For the same purpose, Dr. Hagan also supplied us with three acid-fast organisms isolated from cattle, and one isolated from a case of human leprosy, labeled Kat no. 352. To this collection was added a strain of *M. graminis* obtained through the courtesy of Dr. W. Steenken of the Trudeau Sanatorium. All of these organisms were propagated on Van Tieghem cell mounts, Sabouraud's agar, and glycerin egg medium, and in litmus milk. The organisms were studied both at room temperature and at 37.5°C. Branching, a highly significant characteristic of our organism, was not observed on the cell mounts in the case of *M. graminis*, *M. phlei*, *M. ranac*, the Clegg II bacillus, the cattle isolates, and soil saprophyte no. 135; nor was true branching observed in smears made from litmus milk cultures of these organisms. On the other hand, four of the saprophytic soil strains designated by Dr. Hagan as "without identification"<sup>12</sup> and the Kat no. 352 organism showed abundant and true branching. These branching organisms differ from our organism, however, as follows. Kat no. 352 organism and soil saprophyte no. 121 do not grow on Sabouraud's medium. In addition, the Kat no. 352 strain will grow at 47°C., while the organism considered here resists cultivation at 40°C. and beyond. Soil saprophytes nos. 136 and 127 differ from our organism in that they form a pellicle in broth and produce dry, rough colonies on solid media. Soil saprophyte no. 132 differs from our organism in its scant growth on Sabouraud's agar and its dry, flaky colonies. Further studies of these organisms are in progress. However, since they too are unclassified, identification of the organism here reported with one of these, even though unlikely, would be of no assistance in its ultimate classification.

*Actinomycetes.* The majority of the organisms belonging to the

family Actinomycetaceae may be eliminated from consideration in connection with the identification of our organism because they are not acid-fast. Nevertheless, there is a considerable group of aerobic, slightly acid-fast actinomycetes which grow in the form of branched, vegetative hyphae. These hyphae readily undergo fragmentation and thus give rise to bacillary and coccoid arthrospores. It is to this group of organisms that the generic name *Nocardia* is given. Since the organism under discussion seems most closely allied to this group, the several species of *Nocardia* will be discussed in some detail.

In 1888, Nocard<sup>13</sup> described an aerobic, acid-fast, filamentous organism isolated from farcy of cattle which was identified by Gasperini<sup>14</sup> as *Actinomyces farcinicus*. In 1890, Eppinger<sup>15</sup> described *Cladothrix asteroides*, another aerobic acid-fast actinomycete, as the cause of an infection of the brain and meninges in man. This organism was renamed *Nocardia asteroides* and has been designated as the key species of the genus, *Nocardia*. In 1921, Henrici and Gardner<sup>16</sup> collected reports of 26 instances of human infection by a variety of strains of aerobic acid-fast actinomycetes, *Nocardia*, and were able to group the organisms into three distinct species. The first of these is *N. asteroides*. This organism is characterized by a mealy growth on agar with pale yellow to deep orange colonies; it does not liquefy gelatin or peptonize milk; it is more highly pathogenic for guinea-pigs than for rabbits. The second species is typified by a strain isolated by Birt and Leishman<sup>17</sup> and later named *N. leishmani* by Chalmers and Christopherson.<sup>18</sup> This organism produces a snow-white growth on solid agar, peptonizes milk, and does not liquefy gelatin. It is pathogenic for guinea-pigs and is reported to be pathogenic for rats and rabbits. The third species is that described by Berestneff.<sup>19</sup> It forms a gray to whitish colonial growth on solid media, liquefies gelatin, and is not pathogenic for laboratory animals. To these three species Henrici and Gardner<sup>16</sup> added a fourth, *A. gypsoides*. Henrici<sup>20</sup> later declared this to be not unlike *N. asteroides*.

In addition to the above-described species, five other species of acid-fast actinomycetes which were recovered from infections in man were found to be described in the literature. From a case of pulmonary actinomycosis, Davis,<sup>21</sup> in 1914, observed such an organism in the sputum of a 64-year-old-man, which he described as an "acid-fast streptothrix." He was not able to culture this organism, nor to determine its pathogenicity for animals. In 1920, Vuillemin<sup>22</sup> isolated an acid-fast, Gram-negative actinomycete from a case of bubonic plague and called it *N. jollyi*. Davis and Garcia,<sup>23</sup> in 1923, isolated an acid-fast organism from subcutaneous abscesses on the extremities of a woman. They assigned their organism to the genus *Nocardia*, but did not iden-

tify it fully. Later a group of investigators<sup>24</sup> considered this organism akin to *N. asteroides*. In 1934, Gammel,<sup>25</sup> assisted by Werkman, isolated a new species of acid-fast actinomycete from a human infection and, because of its ability to grow in phenolized media, called it *A. phenotolerans*. In 1937 Goldsworthy<sup>26</sup> described an acid-fast actinomycete which was later considered by Kirby and McNaught<sup>27</sup> to be a variant of *N. asteroides*.

The taxonomic distinction between the acid-fast nocardia and the mycobacteria is difficult because of characteristics held in common by organisms of these two groups. This difficulty was acknowledged by Umbreit<sup>28</sup> in his classification, wherein he set up special groups which he called proactinomycetes. He divided this group into the alpha proactinomycetes which are closely related to the mycobacteria and corynebacteria because of their unstable mycelium in slide cultures, and the beta proactinomycetes which are allied to the true actinomycetes because of their stable mycelium and "actinomycete growth in liquid media." The alpha and beta proactinomycetes are further divided into those which are not acid-fast and are therefore akin to the corynebacteria, and those which are acid-fast and are therefore like the mycobacteria. The organism of this present communication can then be classified as one of the acid-fast beta proactinomycetes.

Rosebury<sup>29</sup> classified the actinomycetes according to their parasitic or saprophytic properties, with a species differentiation based on morphologic, environmental, and cultural characteristics. The organism of this report does not lend itself to identification with one of these groups because it possesses both the properties ascribed by Rosebury to the parasitic type and those belonging to the saprophytic types.

The classification of the actinomycetes most useful to us and, we believe, generally acceptable is that of Waksman and Henrici<sup>5</sup> (1943), which is based on the character of the branching of these organisms. In their classification, the family Actinomycetaceae is divided into aerobic and anaerobic genera. The aerobic groups of organisms, partially acid-fast or non-acid-fast, which form a vegetative mycelium dividing by segmentation into bacillary or coccoid elements (arthrospores<sup>30</sup>) but do not produce conidia, are designated *Nocardia*.

#### TAXONOMIC POSITION OF THE ETIOLOGIC AGENT

The placement of the organism dealt with in this report offers difficulty. That it belongs in one of the above-discussed groups of organisms seems obvious because of its acid-fastness. That it is not *M. leprae*, the organism of Johne's disease, or the organism of rat leprosy is indicated

by the ease with which it is cultivated. The degree of its acid-fastness would tend to place it among the mycobacteria; however, the consistency of its branching and its fragmentation into bacillary and coccoid forms would seem to place it among the nocardia. In addition, its ability to grow on Sabouraud's medium at room temperature would appear to confirm the fungous character of this organism. The conclusion is drawn, therefore, that this organism should be more properly considered a species of *Nocardia*.

In contrasting this organism with the several species of *Nocardia*, as outlined by Henrici and Gardner,<sup>10</sup> sharp differences become apparent. It differs from *N. asteroides*, the type species of this genus, in that it shows a greater degree of acid-fastness, it resists digestion by sodium hydroxide, it produces buttery colonies, it does not produce aerial mycelia, it does not develop other than yellow pigment, it does not form a pellicle in liquid media, it grows poorly on potato agar and gelatin media, and it fails to produce a lesion in rabbits. It differs from *N. leishmani* in that it does not produce a snow-white growth on agar (that is, it forms no aerial mycelia); it does not produce peptonization of milk, and it is not pathogenic for rabbits. It differs from the species reported by Berestneff<sup>19</sup> because it does not produce gray to white growth on solid media and is pathogenic for laboratory animals. It differs from *A. gypsoides* because it does not produce white colonies, it has no proteolytic activity, it does not produce tyrosinase, and is not lethal to laboratory animals. Equally sharp differences between our organism and those strains which are not included in the classification of Henrici and Gardner are apparent. Since the acid-fast streptothrix described by Davis<sup>21</sup> could not be cultured, and its pathogenicity could not be determined, its differentiation from our organism seems obvious. *N. jollyi*, reported by Vuillemin,<sup>22</sup> proved to be Gram-negative, which differentiates it from the organism here reported. *A. phenotolerans*, of Gammel and Werkman,<sup>25</sup> is similar to our organism in that it was extremely acid-fast and capable of growing in a medium to which was added 0.5 per cent phenol, but it differs in that it formed a pellicle in liquid media and produced aerial mycelia.

#### SUMMARY

Inasmuch as this organism is considered a species of *Nocardia*, and inasmuch as there are sharp differences between it and known species of this genus, it is concluded that this organism represents a hitherto undescribed species; for this new species the name *Nocardia intracellularis*, *n. sp.*, is suggested because of the characteristic intracellular

position which it occupies in the human host. A brief characterization in Latin with English translation is as follows:

*Nocardia intracellularis*, *sp. nov.* Filamentis ramosis, in fragmenta secudentibus, ex baculis seriatis constitutis, 0.2-0.45  $\mu$  latis; in culturis in liquoribus colonias ramosas efformat; apice non clavatis; chlamydo sporis non efformantibus; arthrosporibus ellipsoideis apice efformantibus; non-motilibus. Hyphae colorantur per fuchsinam acidospiritu-rectificato haud decolorantur. Difficulter propagatur in gelatina, atque gelatinam non liquefacit. In culturis agar-agar applantis colonias circulares, elevatas, nitentes, leves, non mucosas, post 3-6 dies.

Fluidum hyalinum in culturis liquidis glycerino atque massas, albas, mucosas, ad basem tubuli efformantis. Lac coloratum cum litmo, acidum fit post 20-30 dies. In culturis Sabouraudii vegetat rapide post 3-7 dies. Amylum non vertit. Decompositionem cellulosa non provocat. Tyrosinase non efficit. Indole non efficit. Decompositionem potassae nitrasi non provocat. Non viget in infusionibus tuberorum solani agar-agar. Crescit in jure carnis in quo glycerino atque 0.5 per centum acidum carbolicum adest. In oxygenii absentia non evolvitur sed in 10 per centum carbonei dioxidi viget. Cera origine sola carbonis utor potest. Viget rapide ad temperaturam 37.5°C. Facile evolvitur usque ad temperaturam 40°C.

Habitat: In granulomīs organorum infectorum lymphaticorum observatus est, ac in faecibus homini vivi in quo morbum causat, atque in cadavero in laesionibus granulomatis disseminatis late quas edit.

*Nocardia intracellularis*, *n. sp.* Filaments branched, becoming fragmented, composed of bacillary elements in series, 0.2 to 0.45  $\mu$  in width; in cultures in liquids, branched colonies are formed; not club-shaped at the tips; and lacking chlamydo spores; nonmotile. The hyphae are not at all discolored when stained with fuchsin and treated with acid alcohol. It grows poorly in gelatin, which it does not liquefy. In agar plate cultures the colonies are circular, raised, wet-shining, smooth, and non-mucoid after 3 to 6 days.

In glycerin broth the fluid remains clear; and white, mucoid masses are formed at the bottom of the tube. Litmus milk becomes acid after 20 to 30 days. On Sabouraud's agar it grows well after 3 to 7 days. Starch is not changed. It does not decompose cellulose. Tyrosinase is absent, and indole is not produced. It does not decompose nitrates of potassium. It does not thrive on potato agar. It grows in beef extract broth to which has been added glycerin and 0.5 per cent carbolic acid. It does not develop in the absence of oxygen, but grows in an atmosphere having 10 per cent CO<sub>2</sub>. It can use paraffin as a sole source of carbon. It thrives at 37.5°C. and tolerates well temperatures up to 40°C.

Habitat: Observed in granuloma of infected lymph nodes and in the feces of a living patient whose death it caused. Observed also at autopsy in widely disseminated granulomatous lesions which it produced.

## PATHOGENICITY OF NOCARDIA INTRACELLULARIS

The study of the reaction of animals to inoculation by *Nocardia intracellularis* was divided into three parts: The determination of species susceptibility; a study of the route of infection; and a study of the development of the lesion.

*Species Susceptibility*

In the experiments designed to determine which animals would become infected, chickens, rabbits, goldfish, frogs, rats, mice, and guinea-pigs were used (Table I). The standard inoculum was 0.2 cc. of a suspension of viable organisms in a concentration of 30 organisms per oil-immersion field.

Six chickens were inoculated, 3 by intravenous injection and 3 by intrathoracic injection. They were sacrificed after 10 weeks. Lesions were not found in any of these animals.

Six rabbits were inoculated, 2 by intravenous, 2 by intraperitoneal, and 2 by subcutaneous injection. One rabbit, intravenously inoculated, died 3 weeks later of septicemia, staphylococci being recovered from the blood stream. The site of inoculation showed no reaction. The remaining 5 rabbits were skin-tested and sacrificed after 10 weeks. The average weight gain was 655 gm. Lesions were not found in any of these animals.

Twelve goldfish were used. In 6 the inoculum was injected intraperitoneally, and in 4 it was injected into the dorsal lymph sac. Lesions failed to form in all cases.

Six frogs, *Rana catesbeiana*, were used. In 3 the inoculum was injected intraperitoneally, and in 3 it was injected into the dorsal lymph sac. Lesions failed to form in all cases.

Ten rats were used: 5 were injected subcutaneously and 5 intraperitoneally. Among the group injected subcutaneously there was one which died 1 week later. Its body was not recovered. The remaining 4 were sacrificed 10 weeks later after a negative skin test. These animals showed an average weight gain of 120.9 gm. In one there was proliferation of lymphoid tissue in the spleen, in which acid-fast organisms could be stained. Two other rats showed focal accumulations of epithelioid cells in the lymph nodes. In these foci acid-fast organisms were stained, some of them showing "Y" forms (Fig. 19). The last rat had no lesions. Among the group inoculated by intraperitoneal injection, one rat died of pneumonia in 8 days. Proliferation of macrophages with giant cell formation occurred at the site of inoculation. Acid-fast organisms were stained, some of which were "Y" forms. The remain-

TABLE I  
*Species-Susceptibility to the Organism*

Animal	Route of infection	Weight		Sensitivity	Death	Significant lesions	Organisms stained in tissues
		Initial	Death				
Mouse 1	Subcutaneous	gm. 15.6	gm. 21.3	Negative	Sacr. 72 days	None	No
Mouse 2	Subcutaneous	14.8	21.0	Not done	Died 33 days	Pneumonia, granuloma of lymph nodes	Yes
Mouse 3	Subcutaneous			Negative	Died 40 days	Pneumonia, granuloma of liver	No
Mouse 4	Subcutaneous	14.4	16.7	Negative	Sacr. 72 days	None	Yes
Mouse 5	Subcutaneous	14.3	16.5	Not done	Died 45 days	Pneumonia, focal necrosis of liver	No
Mouse 6	Intraperitoneal	11.4	17.9	Not done	Died 26 days	Granuloma of intestines, lymph node	Yes
Mouse 7	Intraperitoneal	8.4	9.7	Not done	Died 6 days	Focal necrosis of liver, hemorrhage in abdomen	No
Mouse 8	Intraperitoneal	9.4	9.4	Not done	Died 1 day	Peritoneal hemorrhage	No
Mouse 9	Intraperitoneal	13.2	13.2	Not done	Died 2 days	Granuloma, mesenteric lymph nodes	Yes
Mouse 10	Intraperitoneal	12.3	11.6	Negative	Died 34 days	None, focal necrosis of liver	No
Rat 1	Subcutaneous	27.8	26.6	Not done	Died 7 days(?)	No remains	No
Rat 2	Subcutaneous	48.0	191.6	Negative	Sacr. 69 days	Granuloma in spleen	Yes
Rat 3	Subcutaneous	35.0	134.2	Doubtful	Sacr. 69 days	Granuloma of lymph node	No
Rat 4	Subcutaneous	67.2	192.2	Doubtful	Sacr. 69 days	None	Yes
Rat 5	Subcutaneous	44.3	160.2	Negative	Sacr. 69 days	Granuloma of lymph node	No
Rat 6	Intraperitoneal	67.2	192.2	Doubtful	Sacr. 69 days	Granuloma of lymph node	Yes
Rat 7	Intraperitoneal	29.8	182.7	Negative	Sacr. 69 days	Granuloma of lymph node	Yes
Rat 8	Intraperitoneal	46.5	74.8	Not done	Sacr. 69 days	Granuloma at site of inoculation	No
Rat 9	Intraperitoneal	44.9	161.5	Positive	Sacr. 69 days	Granuloma of lymph nodes	Yes
Rat 10	Intraperitoneal	40.3	150	Doubtful	Sacr. 69 days	Granuloma of lymph nodes	No
Guinea-pig 1*	Subcutaneous	575	690	Positive	Died 8 days	Granuloma at site of inoculation	Yes
Guinea-pig 2*	Subcutaneous	380	525	Positive	Sacr. 69 days	Granuloma of lymph nodes	Yes
Guinea-pig 3*	Subcutaneous	350	450	Positive	Sacr. 69 days	Granuloma at site of inoculation	No
Guinea-pig 4*	Subcutaneous	420	575	Positive	Sacr. 70 days	Granuloma, lymph nodes and liver mis, and liver	Yes
					Sacr. 70 days	Granuloma, liver and gastro-intestinal tract	Yes

Guinea-pig 5*	Subcutaneous	535	650	Positive	Sacr. 70 days	Granuloma, epididymis and lymph nodes	Yes
Guinea-pig 6*	Subcutaneous	515	600	Positive	Sacr. 70 days	Granuloma, lymph nodes, liver, and brain	Yes
Rabbit 1908	Intravenous	2175	1575	Not done	Died 21 days	Pneumonia, focal necrosis of liver	No
Rabbit 1911	Intravenous	2325	3150	Negative	Sacr. 68 days	None	No
Rabbit 1913	Intraperitoneal	2025	2900	Negative	Sacr. 68 days	None	No
Rabbit 1919	Intraperitoneal	2275	3100	Negative	Sacr. 68 days	None	No
Rabbit 1922	Subcutaneous	1925	2250	Negative	Sacr. 68 days	None	No
Rabbit 1923	Subcutaneous	2375	2900	Negative	Sacr. 68 days	None	No
Chicken 1	Intravenous				Sacr. 69 days	None	No
Chicken 2	Intravenous				Sacr. 69 days	None	No
Chicken 3	Intravenous				Sacr. 69 days	None	No
Chicken 4	Intrapleural				Sacr. 69 days	None	No
Chicken 5	Intrapleural				Sacr. 69 days	None	No
Chicken 6	Intrapleural				Sacr. 69 days	None	No
Frog 1	Dorsal lymph sac				Died 6 days	None	No
Frog 2	Dorsal lymph sac				Sacr. 60 days	None	No
Frog 3	Dorsal lymph sac				Sacr. 60 days	None	No
Frog 4	Intraperitoneal				Died 20 days	None	No
Frog 5	Intraperitoneal				Sacr. 60 days	None	No
Frog 6	Intraperitoneal				Sacr. 60 days	None	No
Goldfish 1	Intraperitoneal				Died 28 days	None	No
Goldfish 2	Intraperitoneal				Sacr. 92 days	None	No
Goldfish 3	Intraperitoneal				Sacr. 92 days	None	No
Goldfish 4	Intraperitoneal				Sacr. 92 days	None	No
Goldfish 5	Intraperitoneal				Sacr. 92 days	None	No
Goldfish 6	Intraperitoneal				Sacr. 92 days	None	No
Goldfish 7	Tail				Sacr. 92 days	None	No
Goldfish 8	Tail				Sacr. 92 days	None	No
Goldfish 9	Tail				Sacr. 92 days	None	No
Goldfish 10	Tail				Sacr. 92 days	None	No
Goldfish 11	Organisms in water				Sacr. 92 days	None	No
Goldfish 12	Organisms in water				Sacr. 92 days	None	No

\* A moderate swelling appeared at site of inoculation, which receded during the third week.



ing 4 rats were sacrificed after 10 weeks. The average weight gain was 131.9 gm. Two of these animals showed focal accumulations of epithelioid cells in the lymph nodes, from which acid-fast organisms were re-isolated. The other 2 showed no lesions.

Ten mice were used; 5 were injected subcutaneously, and 5 intraperitoneally. In the first group one died at 5 weeks. This animal showed small collections of epithelioid cells in the lymph nodes. Death was due to pneumonia. A second mouse died of pneumonia, also. There were focal collections of macrophages in the lymph nodes with focal necrosis in the liver, but acid-fast organisms could not be demonstrated. A third mouse died after 7 weeks. No lesions were found which could be ascribed to infection by acid-fast organisms. Two mice lived 10 weeks, whereupon they were sacrificed but no lesions were found. The average weight gain was 3.95 gm. Among the group intraperitoneally injected, 2 died by the second day of hemorrhage. In one, acid-fast organisms formed a focal aggregate in a mesenteric lymph node. No other lesions were found. A third mouse died at 6 days of peritoneal hemorrhage. A fourth died at 4 weeks. In this mouse there was macrophagic proliferation in the mesenteric nodes and those surrounding the intestine, with small tubercle-like lesions containing acid-fast organisms in the liver. A fifth mouse died at 5 weeks. No lesions referable to acid-fast organisms were found, but there was focal necrosis in the liver.

Six guinea-pigs were injected subcutaneously. In each a large swelling characterized by an epithelioid reaction of large macrophages and by central necrosis appeared at the site of injection. Acid-fast organisms invariably were isolated in cultures and were stained in histologic sections. Lesions in the guinea-pig differed from those in the patient in that, while the acid-fast organisms usually were arranged in clumps, massive intracellular parasitism was not found. The inguinal lymph nodes in 3 and the tracheobronchial lymph nodes in 4 of the 6 guinea-pigs showed a granulomatous reaction in the form of marked hyperplasia of the macrophages. In these nodes acid-fast organisms were stained. In 4 animals a reaction of the same kind was found in the liver (Fig. 20). These granulomas consisted of an aggregation of macrophages with a central area of necrosis. Inflammatory cells of other types were rare. Three animals showed diffusely scattered areas of focal necrosis of the liver. The lymphoid elements of the gastrointestinal tract showed uniformly a proliferative reaction in which acid-fast organisms were stained in only 2 instances. The lungs also uniformly showed proliferation of the lymphoid elements surrounding the vessels and the bronchi (Fig. 21), but in only one lung were acid-

fast organisms stained. In one guinea-pig there was a characteristic lesion in the wall of the gallbladder, and also lesions appeared in the epididymis (Fig. 22). In one guinea-pig a small group of epithelioid cells was found in the meninges of the basal portion of the brain. In this tubercle-like lesion acid-fast organisms were stained. There was one instance of epithelioid reaction in the spleen in which acid-fast organisms were found; however, in all animals there was a diffuse proliferation of lymphoid elements. Lesions did not appear in the heart, kidney, or adrenal of any inoculated guinea-pig.

### *Route of Infection*

For the purpose of investigating the route of infection, guinea-pigs were selected. Groups of 4 animals were inoculated in each of the following ways: By intravenous, intraperitoneal, and subcutaneous injection; by ingestion; and by instillation into the conjunctival sac (Table II).

In the group inoculated intravenously, the lungs showed proliferation of lymphoid elements in 3 animals, with the formation in one of a tubercle-like lesion carrying acid-fast organisms. One guinea-pig showed no lesion in the lung. Lymph nodes in 2 showed hyperplasia of the reticulo-endothelial elements, but carried no acid-fast organisms. In the livers there were 2 instances of marked reaction in the form of proliferation of macrophages with a central area of necrosis, one of phagocytosis of acid-fast organisms by the Kupffer cells, and one in which there was no lesion. The spleens of these animals showed uniform proliferation of the lymphoid elements, and in one instance acid-fast organisms were seen intracellularly. One of these guinea-pigs showed a granulomatous lesion in the epididymis typical of the kind produced by this organism.

In the group receiving intraperitoneal injections there was uniform hyperplasia of the lymphoid elements in the lungs, lymph nodes, and spleens. In the liver of one of these animals there were groups of epithelioid cells containing scattered acid-fast organisms. In 2 animals the same lesion appeared in the lymph nodes, but the fourth animal showed no lesions, and no acid-fast organisms were stained.

In the group inoculated by subcutaneous injection there was produced uniformly an area of necrosis surrounded by large mononuclear macrophages at the site of inoculation. With the exception of regional inguinal nodes, the lymph nodes were hyperplastic in only one instance. In the livers of 2 animals were found lesions, consisting of large mononuclear phagocytes, with a central area of necrosis. Acid-fast organisms

TABLE II  
Route of Infection

Guinea-pig no.	Route of infection	Weight		Sensitivity	Sacrificed after	Significant lesions	Organisms stained
		Initial	Death				
1876	Intravenous	gm. 700	gm. 625	++	84 days	Granuloma of epididymis	Yes
1890	Intravenous	475	450	++	84 days	Granuloma of lungs and liver	Yes
1882	Intravenous	725	700	++	84 days	Phagocytosis by Kupfer cells and macrophages of spleen	Yes
1895	Intravenous	500	450	++	84 days	Granuloma of liver	Yes
1879	Intraperitoneal	400	600	++	81 days	None	Yes
1892	Intraperitoneal	400	550	++	81 days	Granuloma of lymph node	No
1900	Intraperitoneal	400	675	++	81 days	Phagocytosis of acid-fast organisms in lymph nodes	Yes
1913	Intraperitoneal	325	700	++	81 days	Tuberculous reaction in liver	Yes
1918*	Subcutaneous	650	675	++	84 days	Granuloma of liver	Yes
1924*	Subcutaneous	675	700	++	84 days	Healing lesion at site of inoculation	Yes
1901*	Subcutaneous	725	925	++	84 days	Granuloma of liver	Yes
1902*	Subcutaneous	300	400	++	84 days	Acid-fast organisms stained in liver	No
1906	Ingestion	625	725	++	82 days	Granuloma of lungs and lymph nodes	Yes
1921	Ingestion	350	525	++	82 days	Acid-fast organisms stained in lungs, liver, and lymph nodes	Yes
1905	Ingestion	400	550	++	82 days	None	Yes
1903	Ingestion	425	500	++	82 days	Granuloma of lymph node	Yes
1907	Instillation, eye	500	650	?	85 days	Granuloma of lungs, acid-fast organisms stained in spleen	No
1909	Instillation, eye	775	725	++	85 days	None	Yes
1915	Instillation, eye	375	375	++	85 days	Granuloma of lymph node and spleen	No
1920	Instillation, eye	350	460	++	85 days	Granuloma of lymph node and spleen	Yes

\* Showed granulomatous inflammation at site of inoculation. The lesion was healed at autopsy.

were stained in the necrotic areas. The spleen was uniformly hyperplastic, but no acid-fast organisms were found. None of these animals showed lesions in the epididymis.

In all 4 of the animals which had ingested the inoculum the quantity of lymphoid tissue in the lungs was striking. Three showed lymph nodes with extensive proliferation of macrophages, and in 2 areas of proliferation many acid-fast organisms were stained. These organisms did not occupy an intracellular position. The livers of 2 guinea-pigs showed acid-fast organisms within the Kupffer cells, but no granulomatous lesions. Two animals showed extensive proliferation of the lymphoid tissue of the spleen, and in one spleen acid-fast organisms were stained. The intestines of 2 guinea-pigs showed marked proliferation of the lymphoid follicles, and, in one, acid-fast organisms were demonstrated in the follicles. The kidneys of 2 animals showed nodules of epithelioid cells in which acid-fast organisms were found.

Only 2 of the 4 animals which had received instillation of inoculum into the conjunctival sac showed infection. In one there was conspicuous lymphoid proliferation in the lymph nodes and spleen, the latter containing acid-fast organisms. The other, in addition, showed a granulomatous reaction in the lung. This lesion consisted of a marked proliferation of the large macrophages with acid-fast organisms scattered throughout.

All of the animals used in this experiment were inoculated intradermally with a suspension of organisms killed by autoclaving. This inoculation produced a reaction about 24 hours after the injection. The reaction consisted of swelling and induration with a broad area of erythema. In the 2 guinea-pigs which resisted infection by ingestion of the inoculum there was no reaction. Histologically, the sites of reaction consisted of a central zone of necrosis and liquefaction, with polymorphonuclear leukocytes. In the focus of polymorphonuclear leukocytes numerous fragmented, acid-fast granules were demonstrated. Surrounding this zone of polymorphonuclear response there was extensive proliferation of macrophages, some of which contained large numbers of acid-fast granules. There was, in addition, an infiltration by lymphocytes and eosinophils with fibroblastic proliferation.

#### *Development of the Lesion and Course of the Infection*

For the purpose of studying the development of the lesion and course of the infection, 18 mice and 24 guinea-pigs were used. These animals were inoculated subcutaneously with 0.2 cc. of a suspension containing approximately 30 living organisms per oil-immersion field. They were sacrificed, 2 at a time, at weekly intervals (Table III).

TABLE III  
*Course of the Infection \**

Animal	Sensitivity	Death	Significant lesions	Organisms stained in tissues
Guinea-pig A1	Negative	Sacr. 9 days	Healing scar at site of inoculation	No
Guinea-pig A2	Negative	Sacr. 9 days	Healing scar at site of inoculation	No
Guinea-pig A3	Not done	Died 11 days	Pneumonia; healing at site of inoculation	No
Guinea-pig A4	Not done	Died 11 days	Healing at site of inoculation	No
Guinea-pig A5	Not done	Died 11 days	Healing at site of inoculation	No
Guinea-pig A6	Not done	Died 12 days	Pneumonia; healing at site of inoculation	No
Guinea-pig A7	Negative	Sacr. 16 days	Nothing	No
Guinea-pig A8	Negative	Sacr. 16 days	Nothing	No
Guinea-pig A9	Not done	Died 18 days	Pneumonia; healing at site of inoculation	Yes
Guinea-pig A10	Not done	Died 18 days	Pneumonia; healing at site of inoculation	Yes
Guinea-pig A11	Not done	Died 18 days	Pneumonia; healing at site of inoculation	No
Guinea-pig A12	Not done	Died 18 days	Nothing	No
Guinea-pig A13	Not done	Died 21 days	Nothing	No
Guinea-pig A14	Not done	Died 23 days	Pneumonia	No
Guinea-pig A15	Negative	Sacr. 23 days	Nothing	No
Guinea-pig A16	Negative	Sacr. 23 days	Nothing	No
Guinea-pig A17	Negative	Sacr. 30 days	Granuloma of lymph nodes	No
Guinea-pig A18	Negative	Sacr. 30 days	Focal proliferations in lymph nodes	Yes
Guinea-pig A19	Not done	Died 32 days	Pneumonia	No

Guinea-pig A20 Guinea-pig A21	Doubtful ++	Sacr. 37 days Sacr. 37 days	Focal proliferation in lymph nodes Granuloma of lymph nodes and liver	Yes Yes
Guinea-pig A22 Guinea-pig A23 Guinea-pig A24	++ ++ ++	Sacr. 42 days Sacr. 42 days Sacr. 42 days	Granuloma of lymph nodes and necrosis of liver Peculiar macrophagic response in lungs Nothing	Yes No No
Mouse A1 Mouse A2 Mouse A3	Not done Not done Negative	Died 1 day Died 7 days Sacr. 9 days	Necrosis of liver Granuloma of skin at site of inoculation Granuloma of skin at site of inoculation	No Yes Yes
Mouse A4 Mouse A5 Mouse A6	Negative Not done Negative	Sacr. 9 days Died 11 days Sacr. 16 days	Granuloma of skin at site of inoculation Healing skin lesion at site of inoculation Healing skin lesion at site of inoculation and lymph node	Yes No Yes
Mouse A7 Mouse A8 Mouse A9	++ ++ +	Sacr. 16 days Sacr. 23 days Sacr. 23 days	Healing skin lesion at site of inoculation Healing skin lesion at site of inoculation and lymph nodes Healing skin lesion at site of inoculation	No Yes Yes
Mouse A10 Mouse A11 Mouse A12	+ + Not done	Sacr. 30 days Sacr. 30 days Died 33 days	Nothing Nothing Nothing	No No No
Mouse A13 Mouse A14 Mouse A15	++ ++ o	Sacr. 37 days Sacr. 37 days Sacr. 44 days	Nothing Nothing Granuloma of lymph node	No No Yes
Mouse A16 Mouse A17 Mouse A18	+ o ++	Sacr. 44 days Sacr. 51 days Sacr. 51 days	Scars in spleen and lymph node Nothing Nothing	No No No

\* All animals developed a swelling at the site of inoculation. This swelling gradually subsided after about 2 to 3 weeks.

The mice uniformly showed a slight swelling and occasionally ulceration at the site of inoculation (Fig. 23). Biopsy of one of these lesions showed the characteristic reaction, described in the preceding section. In the areas of necrosis were many acid-fast organisms. Mice which were sacrificed on the 11th day showed disappearance of the central area of necrosis and replacement by a granulomatous reaction of a different form, consisting of large mononuclear phagocytes with Langhans' giant cells. Those sacrificed on the 23rd day showed a granulomatous reaction in the liver and spleen. In those sacrificed on the 30th day, the site of inoculation was completely healed, and in its place only scar tissue was found. Internally these mice showed only extensive hyperplasia of the lymphoid elements of the liver and lymph nodes. Acid-fast organisms were not found. In none of the mice sacrificed subsequently were active lesions found, with the exception of one sacrificed on the 44th day. In this mouse there was an extensive macrophagic proliferation of the regional lymph nodes. Acid-fast organisms were found in an intracellular position in these nodes. In one mouse a large area of scar tissue was found in the spleen. This was thought to be a healed lesion. Three of these mice showed healed, tubercle-like lesions in the liver.

The guinea-pigs, like the mice, exhibited a granulomatous reaction at the site of inoculation. Those sacrificed between the 9th and the 18th day showed healing at the site of inoculation. Guinea-pigs sacrificed on the 30th and 37th days showed internal granulomatous reactions; 2 had focal epithelioid proliferation in the lymph nodes. One showed a lesion of this type in the liver, and proliferation of the reticulo-endothelial elements of the lung, which contained acid-fast organisms. In all 4 there was hyperplasia of the lymphoid elements of the spleen. One animal, sacrificed on the 42nd day, showed a granulomatous reaction in a lymph node in which acid-fast organisms were stained. Acid-fast organisms also were stained in the lung, and there were extensive areas of necrosis in the liver. All guinea-pigs sacrificed subsequent to the 37th day were without active lesions.

The course of infection in the experimental animal was different from that in the patient. At the site of inoculation there developed an area of necrosis surrounded by large mononuclear macrophages. This lesion healed within about 3 weeks. Throughout the phase of healing the lesion was characteristically granulomatous, with proliferation of macrophages and formation of giant cells. The organism occasionally would be found in a "Y" form in such lesions. The infec-

tion usually remained localized. However, in many animals small isolated lesions were produced in lungs, liver, spleen, and intestine. In the course of 4 to 5 weeks all lesions healed, and sensitivity of the tuberculin type was produced. The infection did not kill the animal.

The guinea-pigs used in this experiment were tested by intradermal inoculation of organisms killed by heat. No sensitivity developed until the 30th day of the infection. These animals had a slight reaction, and all sacrificed subsequently showed a reaction of the tuberculin type, which became distinct by the 42nd day.

*Summary of the Studies on the Pathogenicity of Nocardia intracellularis, n. sp.*

*Nocardia intracellularis, n. sp.*, was found to be pathogenic\* for guinea-pigs, rats, and mice, but it produced no lesion in rabbits, chickens, frogs, or goldfish. Infection of guinea-pigs by this organism was successful when it was injected subcutaneously, intravenously, and intraperitoneally, and when fed by mouth; but when it was instilled into the conjunctival sac infection was successful in only 50 per cent of animals. The animals for which *N. intracellularis, n. sp.*, is pathogenic uniformly showed a local reaction at the site of inoculation consisting of proliferation of macrophages with necrosis in the center of the lesion but with little intracellular parasitism. Ten per cent of these animals had a systemic distribution of the lesions, chiefly in the liver, lungs, and lymph nodes. These lesions healed after about 6 weeks, at which time the animals exhibited a tuberculin-like skin sensitivity to a heat-killed suspension of the organism.

GENERAL SUMMARY AND DISCUSSION

In summary, the condition described is a new granulomatous disease entity caused by an unusual acid-fast organism. The source of the infection is not known. Inasmuch as the most numerous and the largest lesions were found in the retroperitoneal and mesenteric lymph nodes and in the lymphoid tissue of the intestines, the gastro-intestinal tract is suggested as the portal of entry. The characteristic reaction to the organism consisted of phagocytosis of the pathogen by large mononuclear phagocytes with proliferation of these cells and the production of multinucleated giant cells. The only other basic pathologic process represented was necrosis of a coagulative type. In the absence of poly-

\*For the purpose of this report, pathogenic is defined as "capable of producing a disturbance in function or structure of any organ or part of the body" and does not imply that the disease must be lethal.



morphonuclear leukocytes and even lymphocytic infiltration, this lesion may be regarded as a pure form of granuloma.<sup>31</sup> This granuloma exhibited no consistent morphologic structure such as tubercle formation, but rather presented itself simply as a diffuse proliferation of macrophages.

In spite of its ability to resist the digestive activity of macrophages and to multiply to an extraordinary degree within these cells, the organism appeared to elaborate little that was toxic to its host. The damage done by it seemed to arise solely out of its ability to grow and multiply within reticulo-endothelial cells of the host, which relationship gave rise to an extraordinary and almost unlimited proliferation of these cells. The initial reaction to this organism was phagocytosis, followed by proliferation of the phagocytes. The end-result of this ultimate form of parasitism is death of the host cells.

The major systemic effects seemed to depend upon the specific location of the larger and more numerous lesions. Their position in the lymphatic system of the gastro-intestinal tract produced obstruction of the lymphatics and consequently failure of absorption of fat. This failure was reflected in the engorged state of the lymphatics and had produced, in part, the systemic effect of inanition. In addition to the nutritional disturbance, there may be other factors in the production of inanition. The extreme degree of proliferation of the macrophages and the clinical course suggested a neoplasm. Indeed, had the presence of organisms not been demonstrated, one would have been justified, from the history and gross examination of the lesion, in considering this disease a peculiar form of lymphosarcoma. In view of this superficial similarity to a neoplasm, the other unknown factors in the production of death might be similar to those with cancer.

Placement of this organism within an established classification has been exceedingly difficult. The degree of acid-fastness and its ability to withstand sodium hydroxide digestion have suggested that it is related to the mycobacteria. However, that it branches consistently and early, that it grows on Sabouraud's medium at room temperature, that it utilizes paraffin as a sole source of carbon, and that it grows rapidly and well on a multiplicity of media seem to identify it more closely with the actinomycetes. Our difficulty in choosing between these two groups is in harmony with the generally prevailing uncertainty of the position of acid-fast actinomycetes. Many have considered them to be mycobacteria, and many have considered mycobacteria to be fungi.<sup>32-35</sup> Into this controversy we prefer not to enter, but the organism seems

most closely related to the genus designated *Nocardia*, which for the time being is assigned to the family Actinomycetaceae. Since we have been unable to find a reported species with which it is identical, we have concluded that this organism represents an undescribed species of *Nocardia*. Because its presence within the cells whose reactivity it provokes in its human host is so constant, so typical, and so impressive, we have utilized this characteristic in assigning the name *Nocardia intracellularis*, n. sp., to it.

Lesions have been produced by this organism in guinea-pigs, rats, and mice, but not in rabbits, chickens, frogs, or goldfish. The inoculation lesion consists of a central zone of necrosis in which groups of organisms may be found. This zone is surrounded by macrophages, in which there are very few organisms. On the outer edge of this macrophagic zone is fibroblastic proliferation. In only about 10 per cent of infections is there systemic spread. When found, the systemic lesions are histologically similar to the local lesion. There have been no fatal infections in these animals. The chief difference between the reaction in the experimental animal and in man is the relative paucity of intracellular organisms and the relative abundance of necrosis in the experimental animal.

The organism under consideration has been contrasted with 37 other acid-fast actinomycetes with respect to the lesion which they produce and the animals for which they are pathogenic (Table IV). Thirty of these organisms were isolated from human sources. Nineteen of these were obtained from abscesses, of which 13 occurred in the lungs. Draining sinuses, including those of madura foot, were the source of three of these organisms. One was obtained from a case of postoperative peritonitis. Three were found in pulmonary lesions which were called bronchopneumonia, and one was isolated from "cirrhotic nodules of the lungs." There was one from a case "resembling plague," one from a case of "fibrosis of the spleen," and one from a patient who did not die, but whose x-ray findings suggested tuberculosis.

From this summary of cases it is seen that acid-fast actinomycetes have been isolated from a wide variety of human lesions. The only case said to be granulomatous was that of Gammel,<sup>25</sup> but the lesions which he described were not similar to those of the patient here presented. In fact, we have been unable to find in the literature a description of any case which is essentially similar.

All of the 37 organisms referred to above show a striking variability in pathogenicity for animals. The variation ranged from lethal patho-

Source and Animal Pathogenicity of *Nocardia intracellularis*, n. sp., Contrasted with 37 Reported Acid-Fast Actinomycetes

TABLE IV

Author	Name of organism	Source	Pathogenic for	Nonpathogenic for
Eppinger <sup>15</sup>	<i>A. asteroides</i>	Abscess of lungs and brain	Guinea-pig, rabbit	Mouse
Aoyama and Miyamoto <sup>36</sup>	<i>A. asteroides</i>	Abscess of lungs	Guinea-pig	Fowl, rabbit, mouse
Buchholz <sup>37</sup>	<i>A. asteroides</i>	Abscess of lungs	Not stated	Guinea-pig
Foulerton <sup>34</sup>	<i>A. asteroides</i>	Abscess of lungs	Guinea-pig, rabbit	Mouse
Horst <sup>38</sup>	<i>A. asteroides</i>	Abscess of lungs	Mouse, dog, guinea-pig, rabbit	Dog
Loehlein <sup>39</sup>	<i>A. asteroides</i>	Abscess of heart, lungs, brain	Guinea-pig, rabbit	Pigeon, mouse
MacCallum <sup>40</sup>	<i>A. asteroides</i>	Postoperative peritonitis	Guinea-pig, rabbit	Rabbit
Schabad <sup>41</sup>	<i>A. asteroides</i>	Abscess of lungs and chest wall	Guinea-pig, rabbit	Rat
Stokes <sup>4</sup>	<i>A. asteroides</i>	"Cirrhotic nodules" of lung	Guinea-pig, rabbit	
Birt and Leishman <sup>17</sup>	<i>A. asteroides</i>	Abscess of brain	Dog, monkey, guinea-pig	
Musgrave and Clegg <sup>42</sup>	<i>A. asteroides</i>	Pleuropneumonia	Guinea-pig(?)	
Sabrazès and Rivière <sup>43</sup>	<i>A. asteroides</i>	Abscess of brain	Guinea-pig(?)	
Sabrazès and Rivière <sup>43</sup>	<i>A. asteroides</i>	Case resembling plague	Guinea-pig(?)	
Berestneff <sup>19</sup>	<i>A. asteroides</i>	Abscess of lungs	dog(?), rabbit(?), mouse(?)	
Vuillemin <sup>22</sup>	<i>A. asteroides</i>	Abscess of sinus of ear	Not stated	
Butterfield <sup>44</sup>	<i>A. rivierei</i> (Brumpt)	Acne of face	Not stated	
De Korté <sup>45</sup>	<i>A. jollyi</i>	Fibrosis of spleen	Not stated	
Flexner <sup>46</sup>	<i>A. anaerobies</i>	Bronchopneumonia	Guinea-pig(?)	
Gammel <sup>25</sup>	<i>A. cylindraceus</i>	Cerebral abscess	Guinea-pig	
Gibson <sup>47</sup>	<i>A. pseudotuberculosis</i>	Sputum in case with radiograph similar to tuberculosis	Rabbit, guinea-pig	
Greco <sup>48</sup>	<i>A. plentolobans</i>	Subcutaneous abscess	Guinea-pig	
Henrici and Gardner <sup>16</sup>	<i>A. gibsoni</i>	Lung abscess	None	
Ferré and Faguet <sup>49</sup>	<i>A. somneri</i>	Bronchopneumonia	Guinea-pig, rabbit	
Bernstein <sup>50</sup>	<i>A. gypsoides</i>	Abscesses of lung and skin	Rabbit, mouse	
Davis <sup>21</sup>	<i>A. sabrazesi</i>	Abscess of spinal cord	Rabbit, guinea-pig, rat	
Davis and Garcia <sup>23</sup>	Unnamed	Saliva of horse	Rabbit, guinea-pig	
Goldsworthy <sup>26</sup>	Unnamed			
Ljubimoff <sup>51</sup>	Unnamed			
Scheele and Petruschky <sup>52</sup>	Unnamed			
Ribytkof and Maloletkoff <sup>53</sup>	Unnamed			
De Mello and St. Antonio	Unnamed			
Fernandes <sup>54</sup>	<i>A. chalmersi</i>			

Lombardo-Pellegrino <sup>55</sup>	<i>A. viridis</i>	Soil	Rabbit, guinea-pig, cat	Rabbit, dog, cat, horse
Nocard <sup>13</sup>	<i>A. farcinicus</i>	Chronic suppurative disease of cattle	Guinea-pig	
Rabe <sup>56</sup>	<i>A. canis</i>	Abscess and draining sinus in neck and forepaw of dog	Rabbit, guinea-pig	
Redaelli <sup>57</sup>	<i>A. sanfeliceii</i>	Rat	Guinea-pig, rabbit, rat, dog	Fowl
Silberschmidt <sup>58</sup>	<i>A. caprae</i>	Lung of goat	Rabbit, guinea-pig	Mouse
Trolldenier <sup>59</sup>	Unnamed	Caseopurulent abscess of dog	Guinea-pig, rat, mouse	Rabbit, fowl, frog, goldfish
Cuttino and McCabe	<i>N. intracellularis, n. sp.</i>	Granulomatous lesion in lymph nodes and spleen		

\* Named *A. leishmani* by the authors; later identified as *A. asteroides* by Foulerton.<sup>34</sup>

† Named *A. freeri* by the authors; later identified as *A. asteroides* by the authors.

genicity for all animals tested to nonpathogenicity for any laboratory animal. Here again, none of them reacted exactly like the organism under consideration.

### CONCLUSIONS

A new disease entity in man was characterized clinically by an abdominal mass, malnutrition, and an afebrile course. The morphologic, cultural, and biochemical characteristics of the causative organism, when contrasted with those of other organisms, present significant differences in every instance, justifying the designation of this organism as a new species to which the name *Nocardia intracellularis, n. sp.*, has been given. This organism is assigned to the genus *Nocardia*, family Actinomycetaceae.

In man the disease produced by this organism is a pure form of granulomatous inflammation, characterized by phagocytosis of the pathogen by reticulo-endothelial cells and proliferation of these cells. In the spleen and lymph nodes the proliferation of macrophages is of such proportion as to displace completely the normal structure. There is equilibrium between the organism and its host cell to the extent that massive intracellular parasitism constitutes perhaps the most distinctive feature of the disease. Comparatively little necrosis occurs, and there is no response on the part of polymorphonuclear leukocytes and other inflammatory elements customarily found in the common infectious granulomas.

*Nocardia intracellularis, n. sp.*, produces a nonlethal disease in guinea-pigs, rats, and mice. It produces no lesions in chickens, rabbits, frogs, and goldfish.

In the experimental animal, *Nocardia intracellularis, n. sp.*, provokes a response which

differs sharply from the reaction in man. This difference is to be found in comparatively extensive necrosis and relative paucity of intracellular organisms. The lesions heal rapidly in the experimental animal and are accompanied by the production of sensitivity of the tuberculin type.

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[ *Illustrations follow* ]

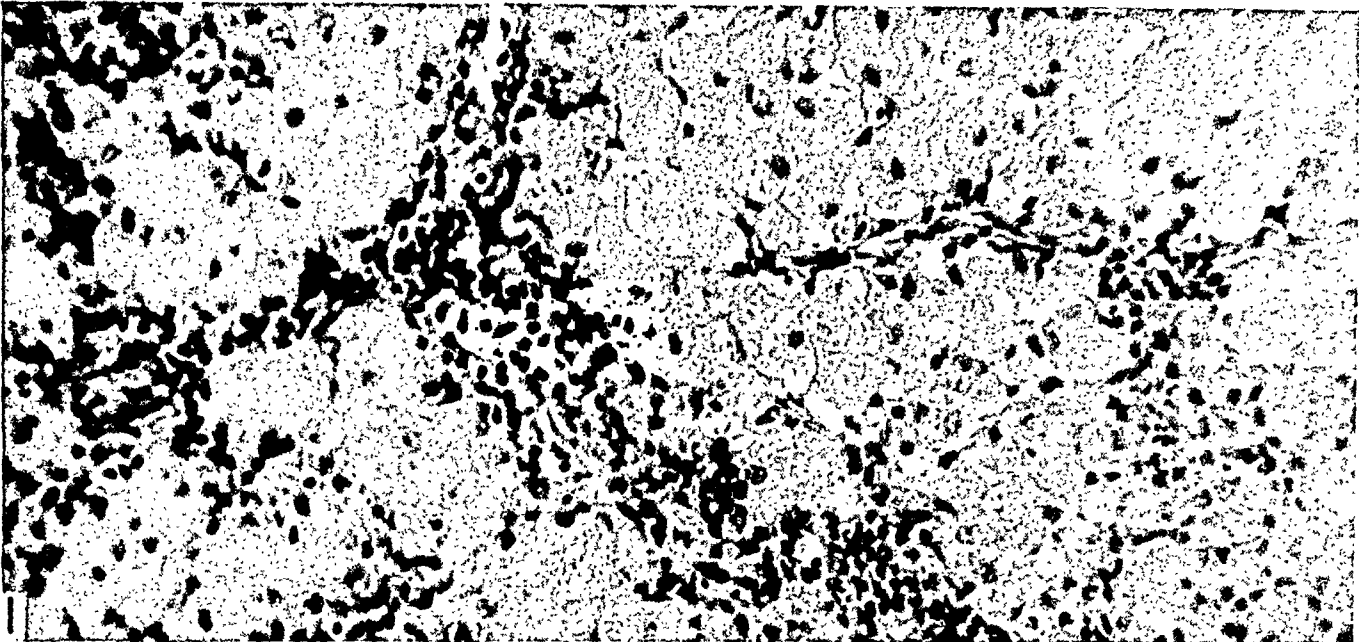


## DESCRIPTION OF PLATES

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### PLATE I

- FIG. 1. From the original lymph node, removed for biopsy, showing widespread replacement of lymphoid tissue by large foamy macrophages. Hematoxylin and eosin stain.  $\times 290$ .
- FIG. 2. Thoracic and abdominal viscera, showing the mass of enlarged and matted lymph nodes of the mesenteric and periaortic groups. There is also enlargement of the left subclavian nodes. The spleen, partially visible at the right, likewise is enlarged. The discoloration of the liver and lungs is an artifact.
- FIG. 3. Colon showing large, irregular ulcers with necrotic bases. The intervening mucosa has lost its normal corrugation. The marginal lymph nodes show enlargement due to proliferation of macrophages.



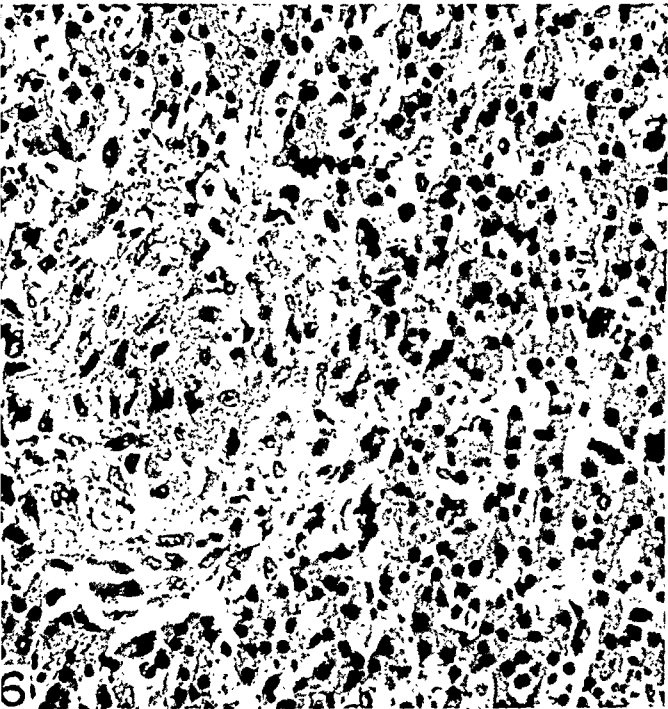
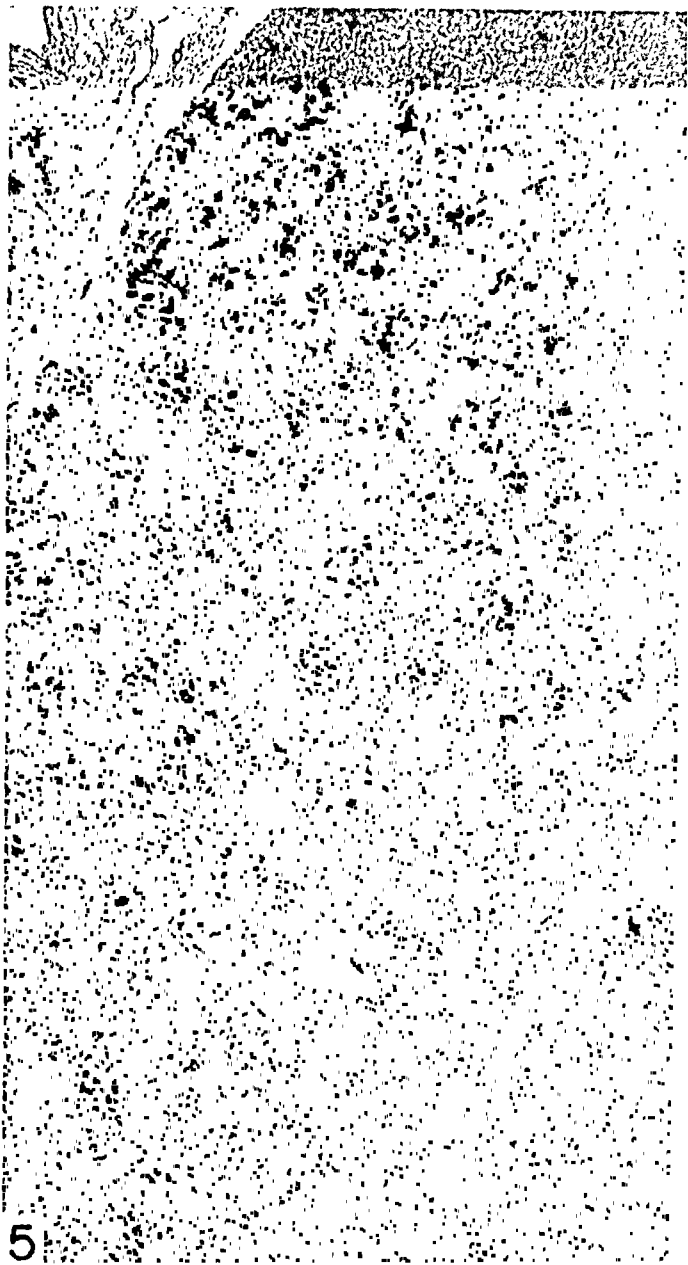
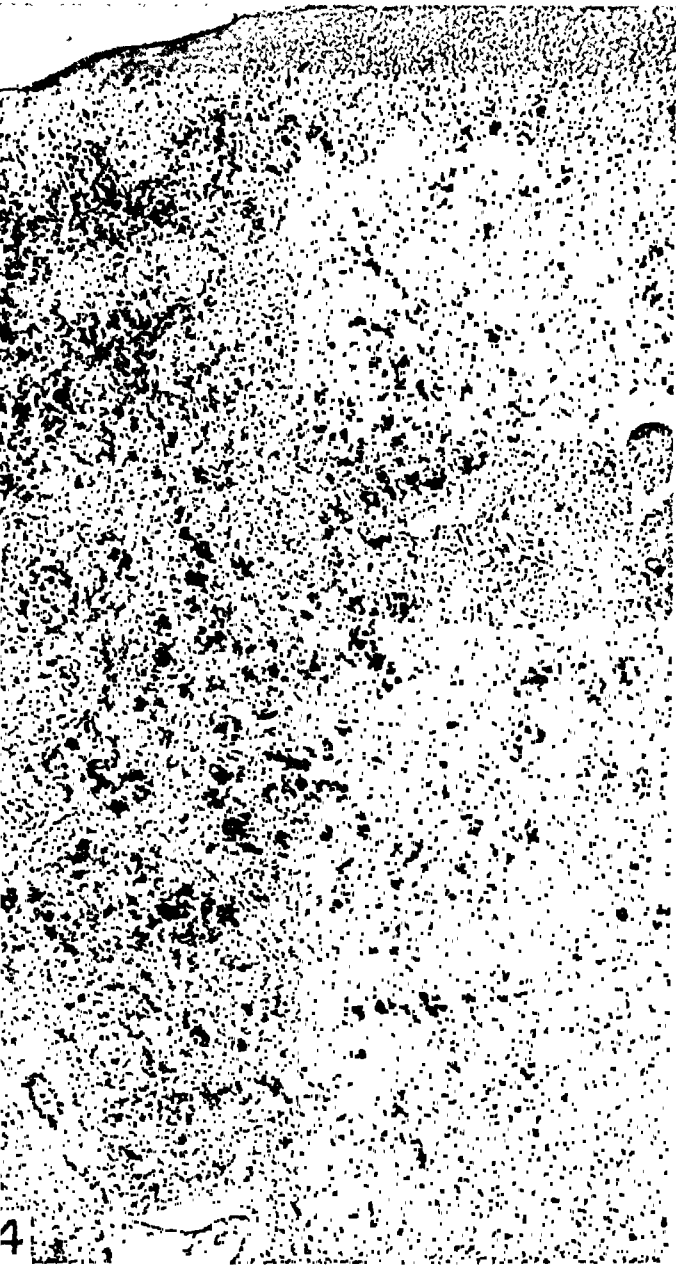
## PLATE 2

FIG. 4. Spleen with complete replacement of its structure by macrophages. The large, deeply stained bodies are multinucleated giant cells. Hematoxylin and eosin stain.  $\times 40$ .

FIG. 5. Mesenteric lymph node with the same changes as were found in the spleen (Fig. 4). In both lymph node and spleen there is absence of necrosis, but the formation of giant cells is more impressive in the lymph node. Hematoxylin and eosin stain.  $\times 40$ .

FIG. 6. A tuberculoid nodule in the liver. There is also enlargement of Kupffer cells. Hematoxylin and eosin stain.  $\times 300$ .

FIG. 7. A higher power view of the same nodule seen in Figure 6. It is stained to show the great number of intracellular, acid-fast organisms, both in cells of the nodule and in isolated Kupffer cells. Ziehl-Neelsen's stain.  $\times 725$ .



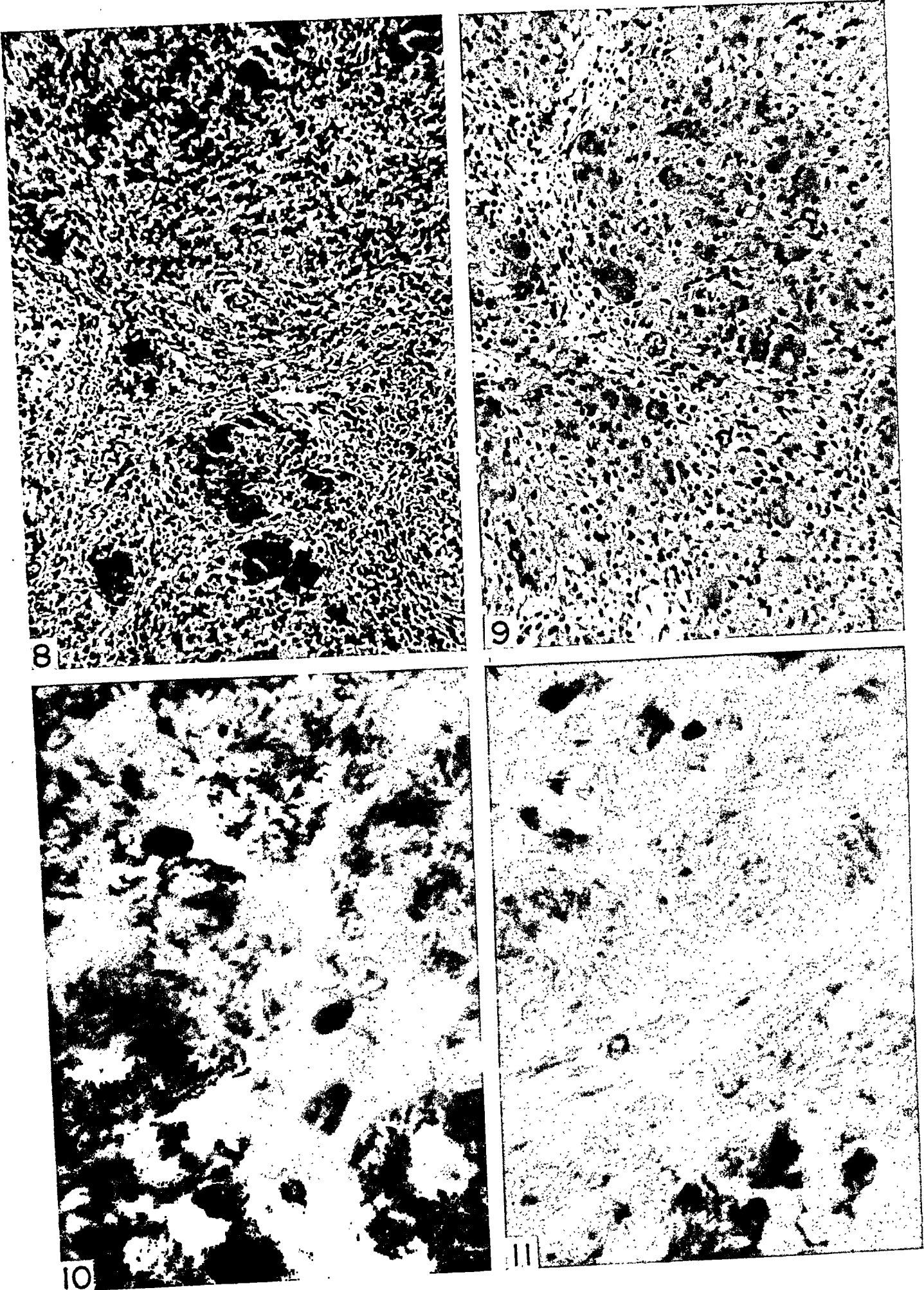
### PLATE 3

FIG. 8. Spleen showing multinucleated giant cells and concentrically arranged epithelioid cells about the central arteries of replaced malpighian corpuscles. Hematoxylin and eosin stain.  $\times 140$ .

FIG. 9. Lymph node showing large mononuclear macrophages and giant cells. Some giant cells have clear, central cytoplasm surrounded by a foamy, peripheral zone. The displacement of normal lymphoid structure is illustrated. Hematoxylin and eosin stain.  $\times 140$ .

FIG. 10. Spleen showing massive, acid-fast, intracellular parasitism of both large mononuclear macrophages and multinucleated giant cells. The absence of organisms in the interstices is a characteristic feature. Ziehl-Neelsen's stain.  $\times 1400$ .

FIG. 11. Lymph node showing two of the giant cells seen in Figure 9. The clear, central zone results from absence of organisms, while in the periphery of the cells they are found in a radiating configuration. The extent of intracellular parasitism by acid-fast organisms is evident. Ziehl-Neelsen's stain.  $\times 750$ .



Granulomatous Nocardiosis

Cuttino and McCabe

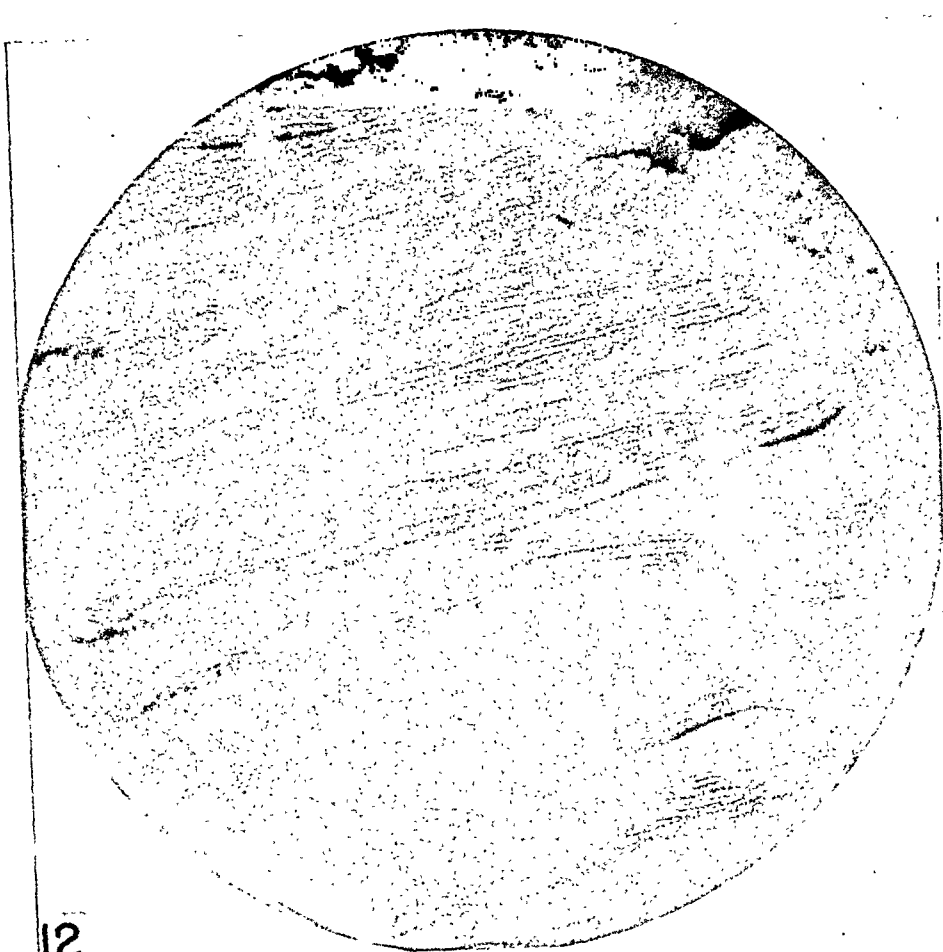
#### PLATE 4

FIG. 12. A 10-day-old streaked plate of the causative organism on glycerin-egg medium, showing the smooth, confluent, moist growth without aerial mycelia.

FIG. 13. A 6-months-old culture on Sabouraud's slant illustrates the vermiform contour of the growth.

FIG. 14. A magnified view of a 12-day-old streak culture on Sabouraud's agar to show the details of colony growth. The individual colonies tend to coalesce and produce the smooth growth seen grossly in Figure 12.  $\times 200$ .

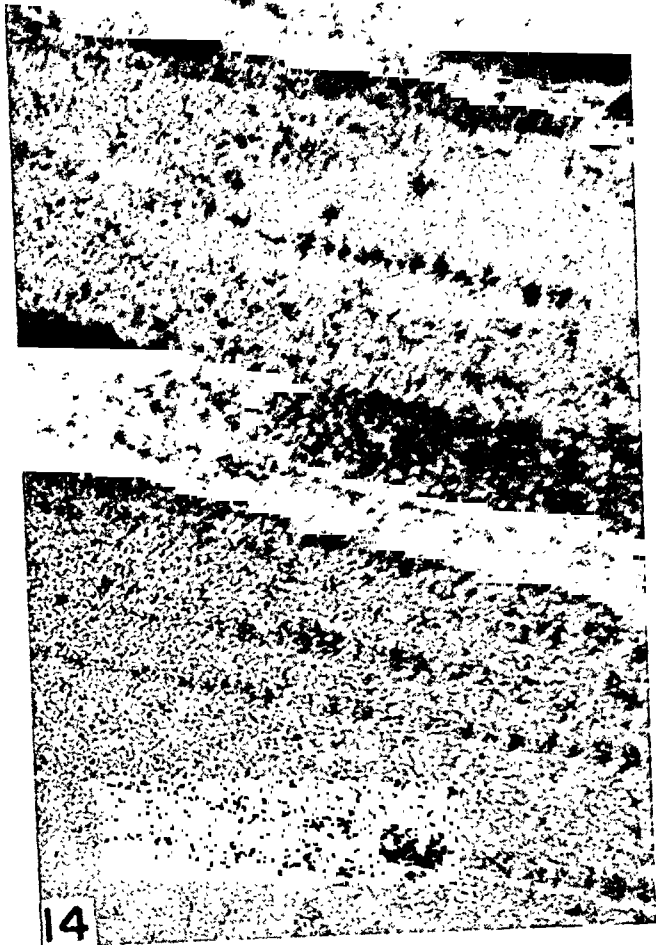
FIG. 15. A stained smear of the organism transferred by loop from Sabouraud's agar slant (Fig. 13). The pleomorphic forms result from the fragmented hyphae. Gram's stain.  $\times 1350$ .



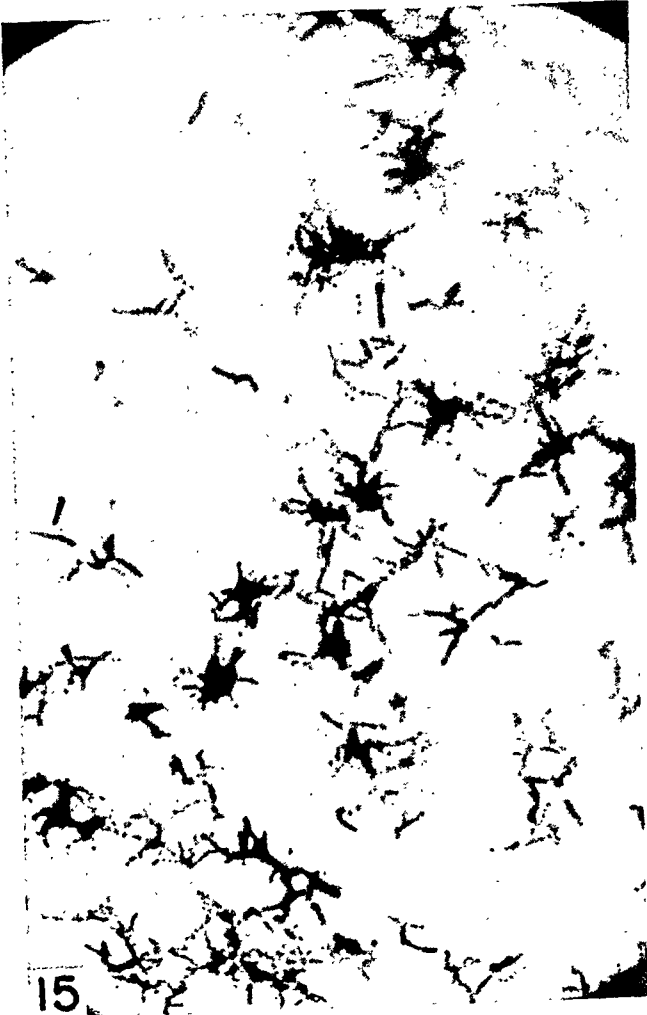
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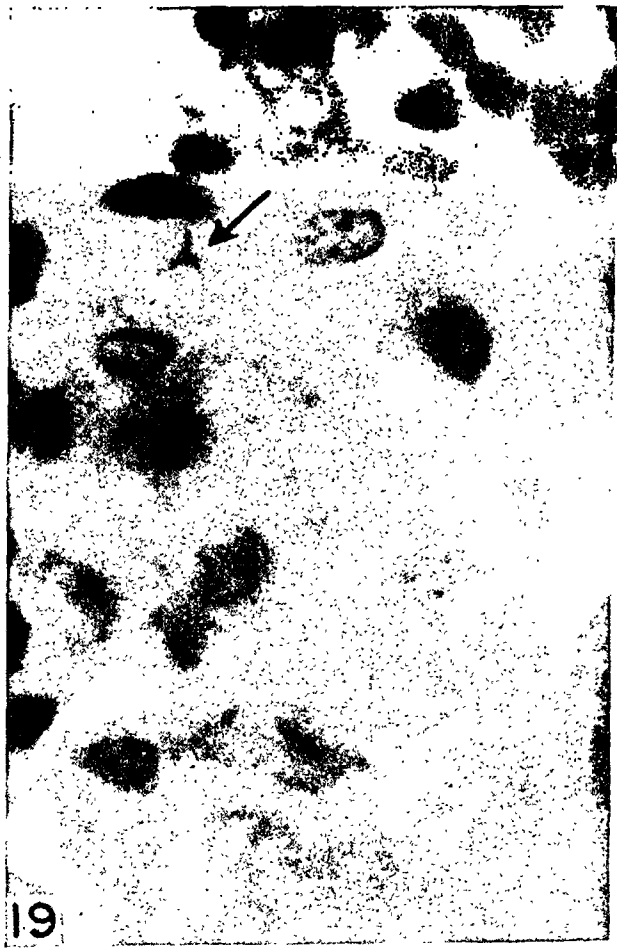
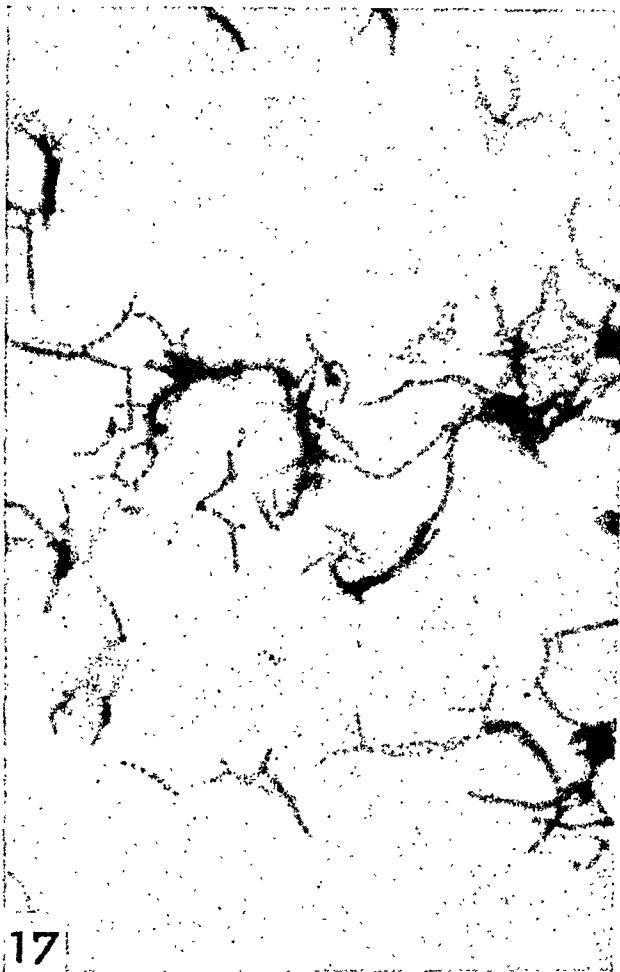
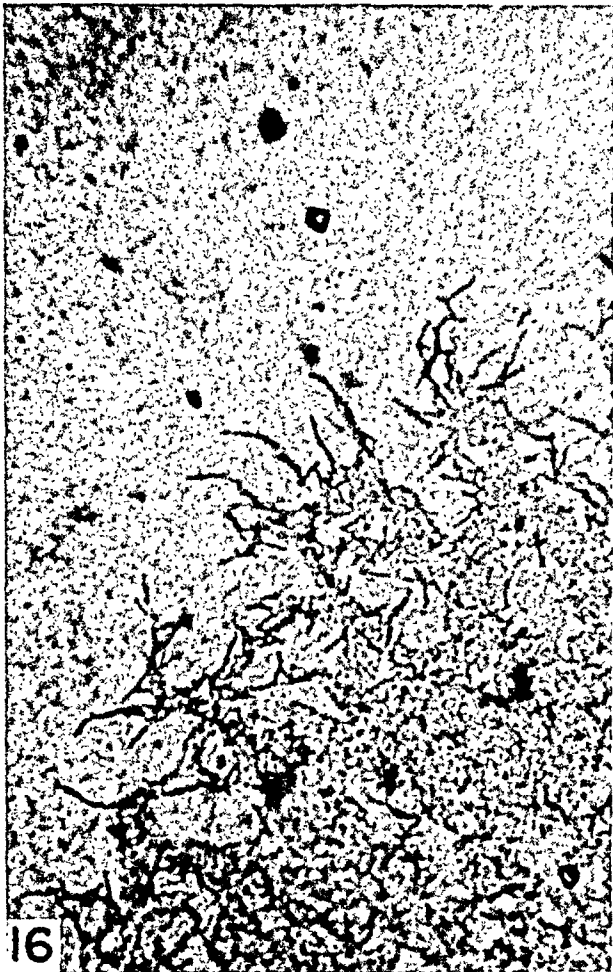


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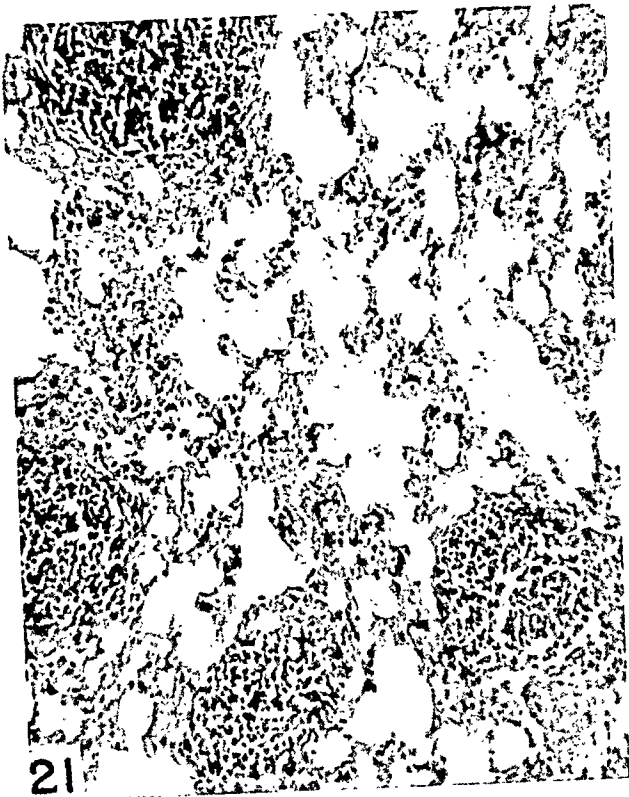
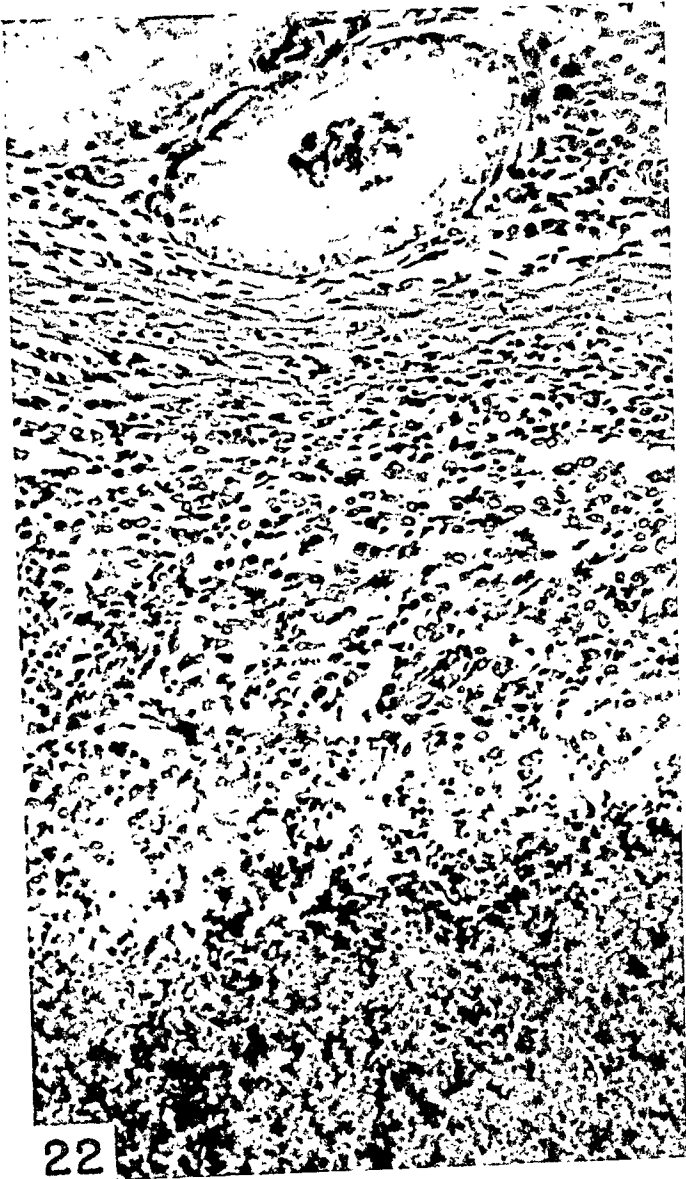
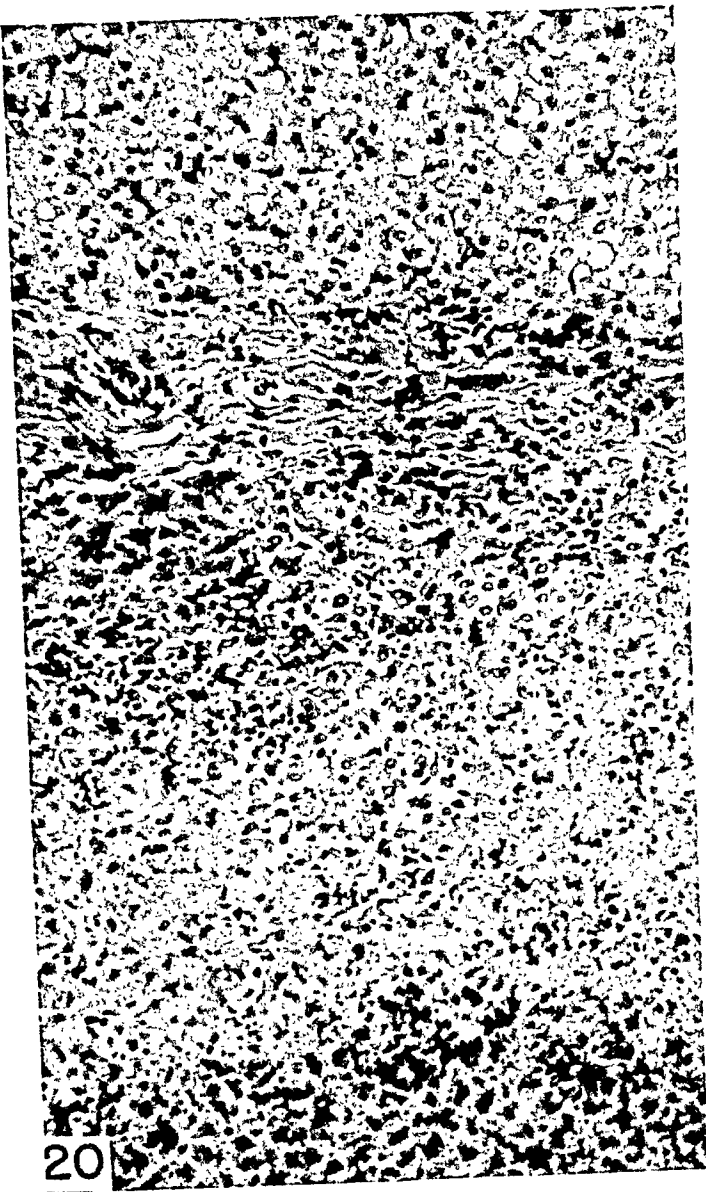
## PLATE 5

- FIG. 16. A photomicrograph of the unstained organism growing on Sabouraud's agar in a Van Tieghem cell mount, showing mycelial growth with true branching.  $\times 800$ .
- FIG. 17. A stained smear of the organism from a 10-day-old litmus milk culture, with preservation of true branching and regularly spaced, spherical to ovoid bodies identified as arthrospores. Kinyoun's acid-fast stain.  $\times 1700$ .
- FIG. 18. Another view of the smear of the organisms from the 10-day-old litmus milk culture (Fig. 17), showing matting of hyphae. At the periphery of the mass may be seen the large pleomorphic, irregularly arranged, nonacid-fast bodies termed granules. Kinyoun's acid-fast stain.  $\times 1350$ .
- FIG. 19. A high-power view of a lymph node of a rat to show the scattered acid-fast organisms in a collection of large mononuclear macrophages. At left is a "Y" form (arrow). Ziehl-Neelsen's stain.  $\times 1700$ .



## PLATE 6

- FIG. 20. Liver of a guinea-pig 4 weeks after subcutaneous injection of viable organisms. In the zone of necrosis (lower), organisms were found in clumps; in the zone of macrophagic proliferation (center), a few isolated intracellular parasites were found. There is a zone of fibrosis separating the lesion from normal liver tissue (above). Hematoxylin and eosin stain.  $\times 150$ .
- FIG. 21. Lungs of a guinea-pig showing enlargement of lymphoid follicles. In only one such instance were organisms stained within these follicles. Hematoxylin and eosin stain.  $\times 150$ .
- FIG. 22. Epididymis of a guinea-pig with necrosis in a central zone surrounded by macrophages as in Figure 20. Many acid-fast organisms were found in clumps in the necrotic tissue. They are fewer in number among the macrophages. Hematoxylin and eosin stain.  $\times 200$ .
- FIG. 23. Two mice showing sites of inoculation after 24 hours (right) and 7 days (left). The 24-hour lesion is a small papule seen at the arrow. The lesion after 7 days is a large ulcer.





# NONLIPID RETICULO-ENDOTHELIOSIS: LETTERER-SIWE'S DISEASE

## A REPORT OF THREE CASES \*

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A clinicopathologic syndrome involving principally the reticulo-endothelial system in infants and young children and having a fatal outcome has come to be known as Letterer-Siwe's disease since the publication of an article by Abt and Denenholz<sup>1</sup> in 1936. In 1942 Green and Farber<sup>2</sup> postulated a possible relationship between Letterer-Siwe's disease, Schüller-Christian's disease, and eosinophilic granuloma of bone. A more general awareness of the disease and its possible relationship to Schüller-Christian's disease and eosinophilic granuloma should stimulate the reporting of additional cases of diseases involving the reticulo-endothelial system. This will aid in the clarification of these disease entities, if indeed they are such, or will serve to classify them properly as variants of a single disease process. With this in mind, three cases of Letterer-Siwe's disease autopsied at this Institute since November, 1940, are reported in detail. These cases fulfill the requirements for Letterer-Siwe's disease as formulated by Abt and Denenholz, namely: splenomegaly, hepatomegaly, generalized lymphadenopathy, localized tumors either over or in bone, secondary anemia, generalized hyperplasia of the cells composing the reticulo-endothelial system, a nonfamilial disease of infants terminating fatally.

## REPORT OF CASES

### Case 1

A 4½-months-old white male infant was admitted to Babies and Childrens Hospital, Cleveland, on November 9, 1940, because of "white patches on the gums and tonsils and a lump on the right side of the head." Except for a cutaneous eruption stated to be present from birth, which had never completely disappeared, the infant was well until the age of 2½ months, when he developed anorexia, "colic," and an otitis media which required bilateral myringotomy. Examination on both occasions revealed cutaneous petechiae and enlarged tonsils. At 4 months, multiple buccal ulcers and a small lump in the right temporal region were noted. The parents and one sibling were alive and well.

Examination on admission revealed a well nourished infant. Over the trunk were many cutaneous lesions, petechiae, and yellow to dark-red papules, many less than 1 mm. in diameter (Fig. 1). Covering the scalp were small, yellow, crusted lesions. In the right temporal region was a subcutaneous, soft mass measuring 1.5 cm. in maximum diameter. Two shallow ulcers were found on the alveolar process of the maxilla. The tonsils were covered by a pale-gray exudate. The liver was palpable 2.5 cm. below the costal margin in the right midclavicular line. Several

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defects in the skull were palpable through the scalp: one, measuring 1 by 1.5 cm., was beneath the mass in the right temporal region; a slightly larger one was present in the right occipital region; and a third was present in the right parietal region.

*Laboratory Examinations.* Blood: red blood cell count, 5,600,000; hemoglobin, 9.1 per cent (Sahli); white blood cell count, 12,000, with 78 polymorphonuclear leukocytes, 20 small lymphocytes, and 2 mononuclear cells per 100 white blood cells; serum cholesterol, 132 mg.; cholesterol esters, 75 mg.; total fat, 656 mg.; and lecithin, 195 mg. per 100 cc. Urine showed a trace of albumin, with 2 or 3 white blood cells per low-power field. Roentgenograms of the skull showed three irregularly rounded, radiolucent areas as follows: One, 1.5 cm. in diameter, in the anterior and inferior portion of the right parietal bone; another, 1 cm. in diameter, in the same bone near the lamboidal suture; and a third, 3 cm. in diameter, in the occipital bone (Fig. 2). The long bones, ribs, and pelvis showed no abnormalities roentgenologically except an area of lessened density at the proximal end of the right humerus on the medial aspect, 0.1 by 0.5 cm. in size. Roentgenograms of the chest showed a peculiar fine "honey combing" of the entire lung fields and a mottled appearance of each apex to the level of the 4th rib.

During the 8 days the infant was in the hospital the temperature remained below 38°C. except on the first day when it rose to 38.9°C. Dyspnea which was noted on the day of admission became progressively worse and was not relieved by administration of intranasal oxygen. On November 16, subcutaneous emphysema of the neck and upper thorax was observed and the infant died later that day. The clinical diagnosis was Schüller-Christian's disease and bilateral chronic suppurative otitis media.

Autopsy (no. 7184) was performed by Dr. F. M. Barry, 3 hours after death.

*Gross Description.* The body was that of a white male infant weighing 6,250 gm. Inspection of the skin and mucous membranes and palpation of the skull and subcutaneous tissues of the neck confirmed the presence of cutaneous and buccal lesions, osseous defects, and emphysema as described previously. The thymus was of normal size, its cut surfaces were lobulated, pale-brown mottled with yellow and flecked with firm, circumscribed, white foci measuring 2 to 3 mm. in diameter. The thyroid weighed 5 gm. The heart weighed 41 gm. (expected weight, 29 gm.) and, except for hypertrophy, was grossly normal. The lungs were pale-pink mottled with gray. On their surfaces were many bullae measuring from 0.1 to 2 cm. in diameter. These had thin, transparent walls and contained air. One along the upper border of the left lung was ruptured and probably was the source of the mediastinal and subcutaneous emphysema. Air spaces measuring 0.1 to 2 cm. in diameter were surrounded by firm septa which were pale pinkish tan mottled with yellow, and measured 0.2 to 0.5 cm. in width, imparting a "honey comb" appearance to the pulmonary parenchyma. The larynx, trachea, and bronchi were normal. The liver weighed 275 gm. (expected weight, 188 gm.), and was grossly normal. The gastro-intestinal tract showed no abnormalities except that the lymphoid tissue of the small and large

intestines was more prominent than usual. The solitary follicles measured up to 0.1 cm. in diameter. The pancreas, adrenals, kidneys, urinary bladder, prostate, and testes were all normally located and showed no lesions grossly. The spleen weighed 30 gm. (expected weight, 16 gm.). Gray follicles, 1 mm. in diameter, surrounded by dark-red pulp were seen on the cut surface. All the lymph nodes throughout the body were enlarged, measuring from 0.5 to 1.5 cm. in diameter. They were pale yellow and firmer than is normal. Their cut surfaces were homogenous and lacked their usual pattern. The brain, which weighed 750 gm. (expected weight, 420 gm.), was grossly normal. The skull bones varied greatly in thickness, in some places being less than 2 mm. There were three osseous defects with smoothly rounded margins, occupying positions as shown by the roentgenograms (Fig. 2). These defects were composed of firm, reddish brown tissue overlying but not adherent to the dura. There was no apparent abnormality of the bones of the orbit, sella turcica, or base of the skull. There was a small perforation in each tympanic membrane. The bone marrow of the sternum, ribs, and vertebrae was reddish brown and of normal consistency.

The microscopic observations on this case and the succeeding two cases will be described together following the gross observations on the third case. The similarity between the lesions of the various organs can be more effectively depicted in this manner.

### *Case 2*

This 8-months-old male infant was seen by a pediatrician 1 week before admission to Babies and Childrens Hospital on June 20, 1945, because of fever and "sores on the gums." The liver and spleen were palpable and a blood count showed moderate leukocytosis. A tentative diagnosis of leukemia was made. The parents stated that at birth the infant had had petechiae over the body and hands which disappeared with the administration of vitamin K. Five weeks before the present illness, a light brown, crusted and scaling eruption appeared over the body and petechiae were noted on the hands, face, and trunk. The infant had been listless for several weeks, he cried when handled, his breathing was more rapid, and his skin paler than usual. The parents and 3 siblings were alive and well.

On admission the child appeared acutely and chronically ill; temperature, 39.9°C.; pulse, 148; and respirations, 60. The skin and mucous membranes were pallid and many petechiae were present over the trunk, being most numerous on the upper-back and back of the head (Fig. 3), but not present on the arms, legs, or feet. Several bullous lesions, 2 cm. in diameter, and containing clear, pale-yellow fluid, were present on the buttocks and inner aspects of the thighs. Elsewhere the skin was dry and scaling. The anterior maxillary, cervical, and axillary lymph nodes were palpable, firm, and discrete. The liver was palpable 4 cm. below the right costal margin and the spleen, 4 cm. below the left costal margin.

*Admission Laboratory Examinations.* Blood: Red blood cell count, 2,400,000; hemoglobin, 38 per cent (Sahli); white blood cell count, 20,500; platelet count, 450,000; 77 polymorphonuclear leukocytes, 20 lymphocytes, 2 mononuclear cells,



and 1 eosinophil per 100 white blood cells; bleeding time, 4 minutes 15 seconds; clotting time, 1 minute 45 seconds; prothrombin time, 80 seconds (normal, 30 seconds); serum protein, 6.1 gm.; cholesterol, 100 mg.; cholesterol esters, 36 mg.; total fat, 716 mg.; lecithin, 209 mg.; calcium, 7.3 mg.; phosphorus, 3.4 mg.; non-protein nitrogen, 35 mg. per 100 cc. of blood; culture, no growth after 6 days; Wassermann and heterophile antibody agglutination tests, negative. Urine: normal. Roentgenograms of the skull and extremities showed no osseous defects. Roentgenograms of the chest showed a widened supracardiac shadow, with increased hilar bronchovascular markings. A splenic puncture showed atypical mononuclear cells suggestive of xanthomatosis (lipidosis).

Throughout his hospital course the patient was acutely ill. Penicillin and sulfadiazine were given without effect on the patient's fever, which showed daily elevations from 38° to 40°C. Supportive measures, consisting of transfusions, hykinone, and ascorbic acid, were used without permanent improvement. The red blood cell count and hemoglobin increased slightly as a result of repeated transfusions, but there was a progressive decrease in the number of white blood cells. The patient lost weight rapidly, with marked wasting of the extremities and edema of the neck and head. He died on the 22nd hospital day. The clinical diagnosis was lipidosis, possible Niemann-Pick's or Gaucher's disease.

Autopsy (no. 8862) was performed by Dr. G. S. Wilson, 6½ hours after death.

*Gross Description.* The body was that of a poorly nourished, white male child weighing 11 kg. The cutaneous lesions of the body and scalp were as described in the clinical abstract. There was hemorrhage into the right leaf of the diaphragm and 60 cc. of blood-tinged fluid in the peritoneum. The thymus, which weighed 13 gm. (expected weight, 18 gm.), was lobulated, reddish brown, and firm. The cut surfaces were reddish brown as compared to the normal pale pinkish or grayish tan. The heart weighed 53 gm. (expected weight, 37 gm.) and, except for size, was normal in all respects. The lungs were uniformly grayish pink and well expanded, but crepitation was decreased throughout. The liver weighed 550 gm. (expected weight, 254 gm.). Sections revealed non-bulging, reddish brown surfaces upon which the lobular pattern was faintly discernible. The esophagus, stomach, small and large intestines, pancreas, adrenals, kidneys, urinary bladder, prostate, and testes were all normally located and not remarkable grossly. The spleen was markedly enlarged, weighing 200 gm. (expected weight, 20 gm.). It was firmer than average, and sections made against increased resistance revealed surfaces which were brownish red flecked with multiple scattered, pale brownish gray foci measuring up to 3 and 4 mm. in diameter. An accessory spleen, measuring 1 cm. in diameter, resembled the spleen in color and consistency. The cervical, axillary, mediastinal, supra-pancreatic, mesenteric, and para-aortic lymph nodes were firm, discrete, and enlarged to approximately three times their normal size. Cut surfaces were flat, firm, and brownish red mottled with gray. The brain

weighed 780 gm. (expected weight, 714 gm.) and was grossly normal. Examination of the skeletal system showed no osseous lesions. The marrow from the sternum, ribs, and lumbar vertebrae was of average consistency and pale brownish red. The skull bones were normal. The middle ear and mastoid air cells showed no evidence of inflammation.

### Case 3

A white male infant was admitted to Babies and Childrens Hospital on December 20, 1945, at the age of 3 weeks because of diarrhea, vomiting, and fever. The infant was born at term of a 23-year-old primipara who was well during her pregnancy. A generalized cutaneous eruption had been present at birth. This was described by a pediatrician as a "polymorphous rash consisting of petechiae, red macules and pale tan scaly crusts." During the first week the eruption cleared spontaneously except for small crusted lesions of the scalp. The infant vomited his feedings frequently and had gained weight slowly. Three days before admission he became irritable and a hoarse cry developed; the following day he had 10 to 12 watery, pale yellow stools. One day before admission a generalized cutaneous eruption again appeared, beginning as petechiae over the lower extremities and progressively spreading over the trunk and becoming macular and papular.

On admission the infant appeared neither acutely nor chronically ill but his cry was hoarse. There was a generalized papular and macular eruption over the entire body except the face, palms of the hands, and soles of the feet. Petechiae were scattered among the other lesions. Yellow crusted lesions were present in the scalp, and red macules on the hard palate. Lymph nodes, except the inguinal, were not palpable; these were small, firm, and discrete. The abdomen was distended and tympanitic but the liver and spleen could be palpated, the liver 5 cm. below the right costal margin, the spleen 4 cm. below the left costal margin.

*Laboratory Examinations.* Blood: Red blood cell count, 3,900,000; hemoglobin, 82 per cent (Sahli); white blood cell count, 6,600, with 50 neutrophilic polymorphonuclear leukocytes, 46 lymphocytes, 2 mononuclear cells, and 2 eosinophils per 100 cells; platelets were present in normal numbers; serum cholesterol, 150 mg.; total fat, 484 mg.; lecithin, 274 mg. per 100 cc. Urine: normal. Roentgenograms of the skull and long bones showed no osseous defects; the lungs were normal. A blood culture taken on the 18th hospital day showed no growth after 6 days.

The patient was given sulfadiazine, 0.5 gm. on admission and 0.125 gm. every 4 hours thereafter for 15 days. He was given Ringer's solution by mouth and parenteral fluids until the diarrhea was under control on the 2nd hospital day. The temperature rose to 39.5°C. on the 2nd and 3rd hospital days, then fell to normal. Diarrhea reappeared on the 8th hospital day and could not be controlled by the usual methods. The weight, which had increased to 3,250 gm., fell to 2,800 gm. and remained there even after marked edema appeared on the 18th hospital day. The lesions of the palate progressed to form confluent, shallow ulcers with gray, firm margins and bleeding bases. On the 19th hospital day the red blood cell count was 1,300,000; the hemoglobin, 38 per cent; white blood cell count, 9,000. Many of the lymphocytes in the blood smear had vacuolated cytoplasm and several blast forms contained vacuoles. The patient died on the 20th hospital day. The clinical diagnosis was reticulo-endotheliosis, probably Letterer-Siwe's disease.

Autopsy (no. 9042) was performed by Dr. A. H. Salans, 3½ hours after death.

*Gross Description.* The body was that of an emaciated white male infant weighing 2.8 kg. Inspection revealed cutaneous and buccal lesions, and edema as noted in the clinical history. The thymus weighed 4.5 gm. (expected weight, 10 gm.). The external and cut surfaces were firm, lobulated, and yellow brown mottled with purplish red. The thyroid weighed 1 gm. and was normal grossly. The heart weighed 17.5 gm. (expected weight, 12 gm.). The right ventricle was slightly dilated; otherwise the heart was normal. The right and left lungs weighed 32 and 26 gm. respectively and, except for slightly reduced crepitation, were normal grossly. The liver weighed 133 gm. (expected weight, 80 gm.). The cut surfaces were nonbulging, deep purple, and without definite markings. The Peyer's patches of the ileum and the solitary lymphoid follicles of the terminal ileum and colon were more prominent than expected. In the latter situation they measured up to 0.2 cm. in diameter and had umbilicated centers. The remainder of the gastrointestinal system was normal grossly. The adrenals and genito-urinary system showed no gross abnormalities. The spleen weighed 22.5 gm. (expected weight, 8 gm.). Cut surfaces showed faintly visible, pale gray follicles surrounded by deep reddish purple tissue. The mesenteric lymph nodes were enlarged, measuring up to 1.5 cm. in diameter. Cut surfaces were pale pink, mottled with gray, circular foci averaging 1 mm. in diameter. The peribronchial and mediastinal lymph nodes were not remarkable. Bone marrow from the lumbar vertebrae was pinkish brown mottled with gray. The autopsy permit did not include examination of the long bones, skull, and brain.

#### MICROSCOPIC OBSERVATIONS

The characteristic cutaneous lesions showed collections of large atypical mononuclear cells and small hemorrhages in the corium. There was parakeratosis with scale formation (Fig. 4) and atrophy of overlying epithelium. A few polymorphonuclear leukocytes were present in the epidermis and corium. In case 2, scaling was not a prominent feature (Fig. 5). The cutaneous lesions in case 3 showed sparsely scattered eosinophils in the corium and atypical mononuclear cells in the subcutaneous fat, in addition to the above changes.

Sections of the thymus showed no recognizable thymic tissue in cases 1 and 2. In case 3 a rare Hassall's corpuscle could be identified. Traversing the gland and dividing it into lobules were narrow bands of fibrous tissue and intermingled fibroblasts. The substance of the gland was composed of loosely arranged cells of several different types. Relatively large cells, approximately 12 to 20  $\mu$ , were predominant in all

cases. These were of irregular outline, with acidophilic cytoplasm and centrally or eccentrically placed vesicular nuclei, which varied in shape, some being oval, other lobulated and grooved, and still others irregularly "crumpled." Chromatin was irregularly distributed in the nuclei and no nucleoli were noted except in case 3. Many of the atypical cells in this case contained large violaceous nucleoli. Mitotic figures were infrequent in each case. The cytoplasm of some of the atypical cells in cases 1 and 2 was vacuolated. There were scattered foci of large multinucleated giant cells having 4 to 12 nuclei and irregular cell borders. Many showed phagocytosis of brown pigment, erythrocytes, and polymorphonuclear leukocytes (Fig. 6). Multinucleated forms were smaller and less numerous in cases 2 and 3. Scattered eosinophils and foci of small lymphocytes were present in each thymus. Portions of each gland were unusually vascular and capillary proliferation was conspicuous. Rare foci of necrosis, approximately one-sixth the diameter of a low-power field, were seen in case 2. Special stains revealed sudanotropic droplets in the cytoplasm of some of the large mononuclear and giant cells, but examination of the tissues under crossed Nicol prisms showed no doubly refractile crystals. Sections treated with hydrochloric acid and ferrocyanide showed a moderate amount of iron pigment within phagocytic cells.

In only the first case did the thyroid show involvement by characteristic lesions composed of large atypical mononuclear cells. Foci of these cells formed nodules in the interlobular connective tissue and in some instances projected into acini.

Sections of the heart and aorta were normal in all cases except the first, which showed small hemorrhages in the myocardium and endocardium.

The amount of pulmonary tissue involved by reticulo-endothelial cells and the degree of bronchopneumonia varied in the three cases. In case 1 the changes were predominantly interstitial. Alveolar walls were thickened by virtue of an increase in the cellular constituents. These cells were principally of the type previously described. Atypical mononuclear cells were free also within alveoli; an occasional cell contained sudanotropic droplets. A few lymphocytes and neutrophilic and eosinophilic polymorphonuclear leukocytes were admixed with the atypical mononuclear cells. Interlobular septa, peribronchial and perivascular tissue, and included portions of the pleura contained similar cells in variable numbers. Some were observed within lumina of blood vessels. Emphysema was conspicuous in some sections and there was a small focus of bronchopneumonia in the left lower lobe. In case 2 the

interstitial changes, while similar, were less profound than in the previous case. There was a small focus of bronchopneumonia in the right middle lobe. The last case showed the least interstitial involvement by large atypical mononuclear cells. A section from the right upper lobe showed a nodule composed chiefly of these cells (Fig. 7). Sections of the larynx and trachea in this case showed many fibroblasts in the subepithelial connective tissue and scattered foci of polymorphonuclear cells.

Except for diffuse cloudy swelling of hepatic cells and fatty metamorphosis, changes in the liver in case 1 were slight. A few Kupffer cells were enlarged and there was a slight increase of connective tissue in the portal regions. A small nodule of atypical mononuclear cells was present within the wall of a bile duct. In cases 2 and 3, many Kupffer cells were enlarged and showed phagocytosis of polymorphonuclear leukocytes and erythrocytes. The connective tissue of the portal regions was greatly increased and contained many large atypical mononuclear cells, some of these forming nodules of 12 to 16 cells. Foci of hematopoiesis were numerous in case 3. Eosinophils not confined to these foci numbered 12 to 15 per high-power field. Several foci of atypical mononuclear cells were present in the interlobular connective tissue of the pancreas in the first and second case.

A section taken through the gingival ulcer in the first case showed a layer of large atypical mononuclear cells of the type seen elsewhere, covered by neutrophilic polymorphonuclear leukocytes mixed with fibrin. The atypical cells were grouped in nodules. Some of the cells contained mitotic figures and some bizarre nuclei (Fig. 8). Sections from the remaining portions of the gastro-intestinal tract in each case differed only in degree of involvement of the interglandular and lymphoid tissue by atypical cells. This was less in the stomach and jejunum. The lymphoid follicles and Peyer's patches were partially replaced by large mononuclear cells (Fig. 9). Mitotic figures were observed in every 2 or 3 high-power fields. Eosinophils numbered 6 to 10 per high-power field. These changes were most profound in case 3 in which the mucosa of the jejunum, ileum, and colon was increased two to three times its normal thickness because of the large number of atypical cells in the interglandular stroma. The mucosa over the Peyer's patches and lymphoid follicles was ulcerated.

Diffusely scattered throughout the loose peripelvic connective tissue of the kidneys were large atypical mononuclear cells. In addition there was a small circumscribed nodule of similar cells in the cortex of the right kidney in case 2. In this case sections from the testes showed numerous atypical cells in the connective tissue underlying the tunica

vaginalis and in the interlobular septa; in the latter situation they occasionally formed nodules of 15 to 20 cells. The testes in the other two cases showed no abnormal changes.

In case 3 the medullary cells of the adrenal were replaced mostly by atypical mononuclear cells mixed with a few eosinophils. Similar cells were present in the pericapsular fat and some of these exhibited phagocytosis of erythrocytes.

In cases 2 and 3 the normal pattern of the spleen was considerably altered by the presence of large numbers of atypical mononuclear cells. In case 1 the general pattern was retained even though nodules composed of 12 to 30 large mononuclear cells formed the centers of most of the follicles (Fig. 10). In all cases there was endothelial hyperplasia of central arterioles and sinuses. Phagocytosis of erythrocytes and of brown pigment was conspicuous, particularly in cases 1 and 2.

Sections from many lymph nodes in all cases were similar but varied considerably in degree of involvement. In case 1 the general microscopic appearance was similar to that in the thymus except that giant multinucleated cells were scarce. In case 2 the normal pattern was more or less well preserved, with an increase of large mononuclear cells in the central portions and peripheral sinuses. Phagocytosis of polymorphonuclear leukocytes and erythrocytes by the reticulo-endothelial cells in the para-aortic lymph nodes was conspicuous. Many of these cells had vacuolated cytoplasm (Fig. 11). In case 3, eosinophils were relatively numerous. One lymph node from the mesenteric group contained several minute abscesses surrounded by mononuclear cells. In the tonsils of case 1 the lymphoid tissue was replaced by nodules of atypical mononuclear cells, which penetrated deeply into the underlying tissue and in some foci extended into striated muscle. No sudanotropic droplets were demonstrated in the reticulo-endothelial cells of the spleen, lymph nodes, or tonsils in case 1, but they were present in the vacuolated cells of the para-aortic lymph nodes in case 2 and in scattered mononuclear cells in lymph nodes, pulmonary alveoli, and cutaneous lesions in case 3.

Special stains showed inconstant amounts of reticulin where the reticulo-endothelial cells were most numerous. In some instances fibrils of reticulin were in intimate association with these cells. In other sites a small amount of reticulin surrounded foci or nodules of these cells in the spleen and lymph nodes but the fibrils did not extend among the cells. None was observed in association with the large mononuclear cells of the cutis in case 3, yet there was a heavy reticulin network in the pulmonary nodule composed of these cells in the same case.

A section through one of the cranial defects in case 1 revealed dense

fibrous connective tissue replacing the bone. Foci of cells, similar to those described as reticulo-endothelial elsewhere, were present. Adjacent to this defect the usual two tables of compact bone were present but the bone marrow contained many foci of large, irregularly shaped reticulo-endothelial cells. Bone marrow from the sternum in case 2 and from a lumbar vertebra in case 3 showed a few large atypical mononuclear cells among the normal cellular components.

The anatomic diagnoses in case 1 were: Reticulo-endotheliosis (non-lipid) of skin, gums, thymus, thyroid, lungs, liver, pancreas, lymphoid tissue of gastro-intestinal tract, pelvis of kidneys, spleen, lymph nodes, tonsils, bones of skull, and bone marrow; bronchopneumonia of lower lobe of left lung; emphysema of lungs; emphysema of tissues of anterior mediastinum and subcutaneous tissues of face, neck, and upper anterior thorax; fatty metamorphosis of liver; perforations of tympanic membranes (recent bilateral myringotomy).

The anatomic diagnoses in case 2 were: Nonlipid reticulo-endotheliosis (Letterer-Siwe's disease) involving skin, thymus, lungs, liver, pancreas, lymphoid tissue of intestines, kidneys and peripelvic fat, testes, pars nervosa of pituitary body, spleen, lymph nodes, and bone marrow; bronchopneumonia of middle lobe of right lung; recent hemorrhage into right leaf of diaphragm; hemoperitoneum.

The anatomic diagnoses in case 3 were: Nonlipid reticulo-endotheliosis (Letterer-Siwe's disease) involving skin, thymus, larynx, liver, lymphoid tissue of intestines, spleen, lymph nodes, bone marrow, adrenals, and periadrenal and subcutaneous fat; bronchopneumonia; hematopoiesis of liver; edema of peri-orbital tissues, hands, and feet.

#### COMMENTS

The remarkable similarity of the clinical courses and of the anatomic and microscopic observations in the foregoing cases leaves no doubt that they belong in the category of reticulo-endotheliosis known as Letterer-Siwe's disease. All showed a cutaneous eruption, pronounced hepatomegaly and splenomegaly, and variable degrees of lymphadenopathy at the time of hospitalization. Other presenting symptoms (mass over the right temporal bone in case 1, buccal ulcers in cases 1 and 2, diarrhea and hoarseness in case 3) may all be explained by localized proliferation of reticulo-endothelial cells. The course of the disease in all three instances was rapid and accompanied by a progressive anemia in cases 2 and 3. Only on admission was a blood count done on case 1, and this showed no anemia, but anemia probably did develop later. The fatal termination of these cases is in accord with other reports of this

TABLE I  
Summary of Pertinent Observations on the Clinical Course, Physical and Laboratory Examination

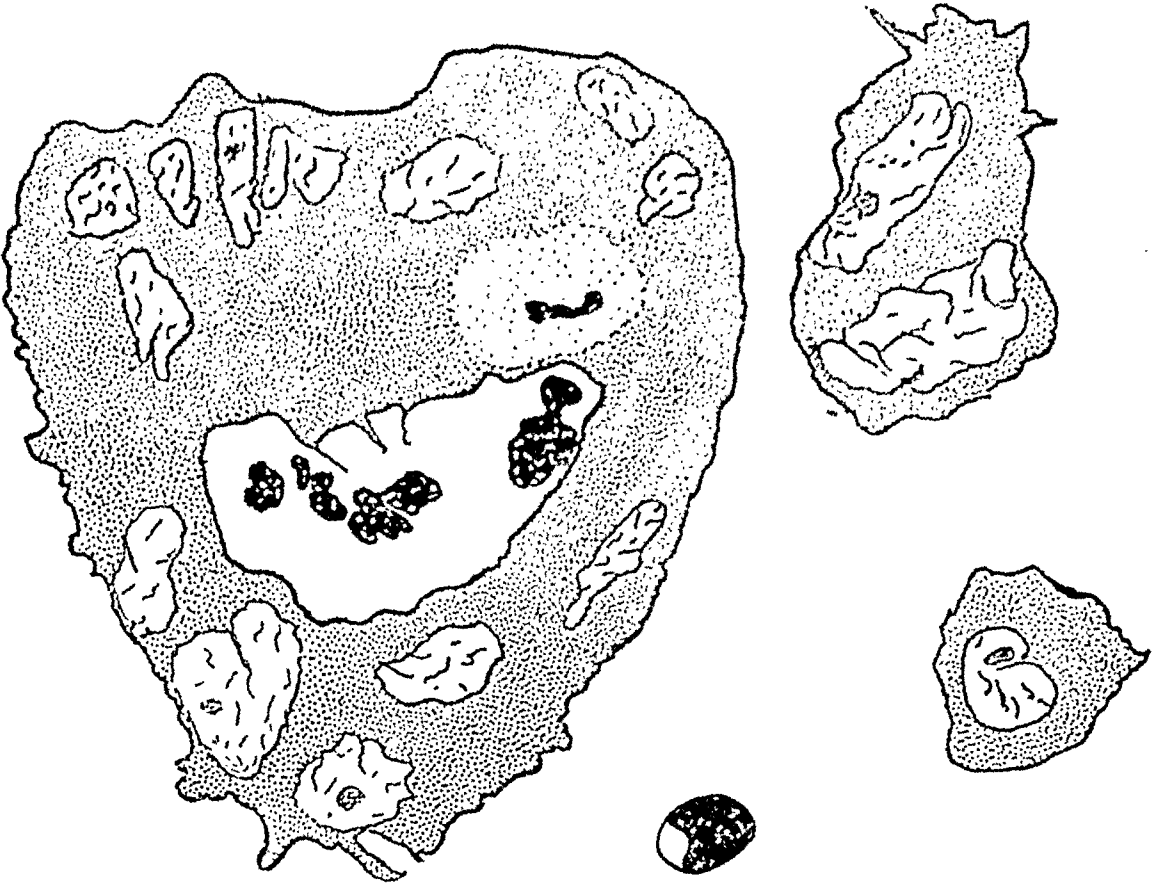
	Case 1	Case 2	Case 3
Sex and race	Male, Gentle	Male, Gentle	Male, Gentle
Age	4½ months	8 months	3 weeks
Presenting symptom	"Lump," right side of head	Pallor, "buccal ulcers"	Diarrhea
Fever	38.9°C.	39.0°C.	39.5°C.
Liver	Palpable	Palpable	Palpable
Spleen	Not palpable	Palpable	Palpable
Lymph nodes	Not palpable	Axillary, cervical, maxillary: palpable	Inguinal: palpable
Cutaneous eruption	From birth	Petechiae at birth	Polymorphous rash at birth
Osseous lesions	Multiple defects in skull, defect in proximal end of right humerus	None by x-ray	None by x-ray
Red blood cell count	5,600,000 (hemoglobin, 94%)	2,400,000 (hemoglobin, 38%)	3,900,000 (hemoglobin, 82%)
White blood cell count	12,000	20,500	6,600
Platelets	Not recorded	450,000	Normal numbers
Serum cholesterol	132 mg./100 cc.	100-135 mg./100 cc.	150 mg./100 cc.
Serum lecithin	195 mg./100 cc.	206 mg./100 cc.	274 mg./100 cc.
Total fats	656 mg./100 cc.	716 mg./100 cc.	484 mg./100 cc.
Duration	Approximately 2 months	3-4 weeks	23 days

condition. Table I summarizes the pertinent observations relative to the clinical courses, physical and laboratory examinations.

In all cases the anatomic and morphologic features were similar. There was universal involvement of the cells of the reticulo-endothelial system. The marked proliferation or hyperplasia of these cells resulted in an increase in the size of those organs having a conspicuous reticulo-endothelial component. This hyperplasia was both diffuse and focal. In the latter instance it resulted in the formation of nodules. While the individual cells varied slightly in size from case to case and sometimes from organ to organ in the same case, they were strikingly uniform in other respects. Text-Figure 1 is a camera lucida drawing of typical cells from the thymus in case 1, which shows some of the salient characters of these cells. Text-Figure 2 is



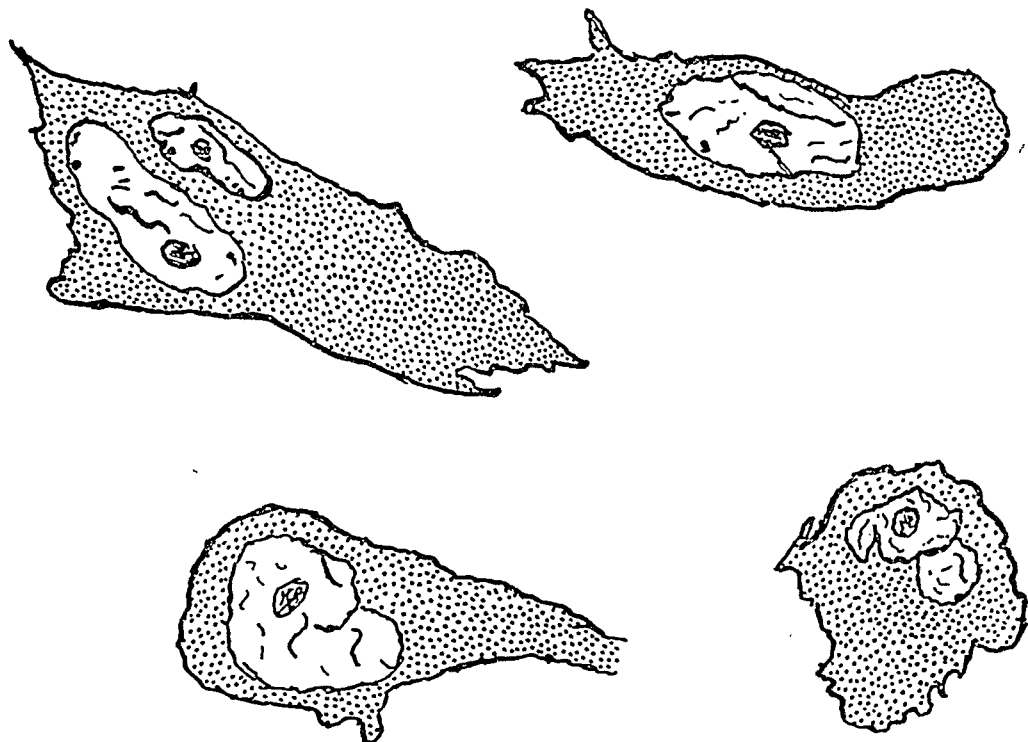
a camera lucida drawing of typical cells seen in the spleen in the same case. Another noteworthy feature was the endothelial and fibroblastic proliferation observed in some of the granulomatous lesions of certain organs, principally, the thymus, lungs, and spleen.



Text-Figure 1. Camera lucida drawing of typical cells seen in the thymus in case 1. The giant cell contains phagocytized leukocytes. A clear halo surrounds the nuclear remnants of several of these cells. The small cell with a dark nucleus in lower center field is a lymphocyte, for comparison of the relative size of the cells.

Even though cutaneous lesions differed slightly on gross examination in the three cases, the microscopic appearance was strikingly similar (Figs. 4 and 5). Few organs escaped involvement by reticulo-endothelial hyperplasia. The thymus showed the most striking changes, being unrecognizable on microscopic examination alone in cases 1 and 2. The significance of these constant and profound changes is not known. The lungs, liver, spleen, and lymphoid tissue were constantly but variably involved. The lungs showed the most profound changes in case 1. The degree of hepatic involvement varied considerably, and microscopically did not seem sufficient to cause hepatomegaly. Splenic involvement resulted in splenomegaly. In case 2, the spleen was ten times the expected size. The lymph nodes and the lymphoid tissue of the gastrointestinal tract showed similar changes. This involvement did not result

in marked lymphadenopathy, nor in unusual increase in size of the solitary follicles and Peyer's patches of the intestines except in case 3. The endocrine glands were not universally involved by reticulo-endothelial proliferation. The only glands so involved were the thyroid in case 1, the testes and pituitary body in case 2, and the adrenals in case 3. The bone marrow showed slight to moderate involvement in all instances, with groups of reticulo-endothelial cells replacing the normal cells of the bone marrow. This change, if extensive, may be a factor in the production of anemia of the progressive nonregenerative type which occurs in Letterer-Siwe's disease. Only in case 1 did the changes produce osseous defects, visible on roentgenographic examination.



Text-Figure 2. Camera lucida drawing of typical cells from the spleen in case 1. Of note are the irregular cell outline and large vesicular nuclei.

While phagocytic activity of the reticulo-endothelial cells was observed in all cases, it was particularly profound in cases 1 and 3. This property of the reticulo-endothelial cells might explain the presence of lipid in some of the cells. In case 3 the phagocytic activity was concerned chiefly with the ingestion of erythrocytes. If pronounced, this activity may be an additional factor in the production of anemia. Another noteworthy microscopic feature was the presence of mitotic figures in the foci of hyperplasia. This observation has not been uniformly reported.

#### REVIEW OF LITERATURE

In 1936 Abt and Denenholz<sup>1</sup> reported a case of nonlipid reticulo-endotheliosis in an infant, which fulfilled the criteria established by

Siwe<sup>3</sup> in 1933. The first case was reported by Letterer,<sup>4</sup> in 1924, as proliferative aleukemic reticulosis. The second and third cases were those of Akiba<sup>5</sup> and Guizetti<sup>6</sup> reported in 1926 and 1931. Siwe reported the fourth case and reviewed the 3 previous cases. Abt and Denenholz included in their series the cases of Podvinec and Terplan<sup>7</sup> (1931), Gittins' case 4 (1933), and the cases of Foot and Olcott<sup>9</sup> (1934), and of Roussy and Oberling<sup>10</sup> (1934). These cases are ably summarized by them<sup>1</sup> (Table 2, page 506). A case by van Creveld and Ter Poorten,<sup>11</sup> reported in 1935, was not accepted as a noninfectious reticulo-endotheliosis because the infant had had an otitis media from the age of 4 weeks. This case had defects of the membranous bones of the skull and cystic lesions of the humeri and ribs. Abt and Denenholz also excluded a case by Schultz, Wermbter, and Puhl.<sup>12</sup> Siwe believed this to be an atypical case of Schüller-Christian's disease and it was similarly classified by Abt and Denenholz.

In September, 1940, Wallgren<sup>13</sup> reported 2 cases and reviewed 15 cases. He included those reported by Abt and Denenholz<sup>1</sup> and added several which had been reported as infectious nonlipid reticulo-endotheliosis. The cases of Schultz, Wermbter, and Puhl<sup>12</sup> (1924), Krahn<sup>14</sup> (1926), Sherman<sup>15</sup> (1929), Gittins' case 2<sup>8</sup> (1933), Uher<sup>16</sup> (1933), Klostermeyer<sup>17</sup> (1934), and of van Creveld and Ter Poorten<sup>11</sup> (1935) were regarded by Wallgren as infectious nonlipid reticulo-endotheliosis. The cases of Podvinec and Terplan,<sup>7</sup> and of Foot and Olcott,<sup>9</sup> cited by Abt and Denenholz as noninfectious nonlipid reticulo-endotheliosis, were thought by Wallgren to be infectious reticulo-endotheliosis. It is interesting to note that Akiba<sup>5</sup> regarded his own case as being related to an infection, yet both Wallgren, and Abt and Denenholz classified it as a noninfectious reticulo-endotheliosis. Similarly, Letterer,<sup>4</sup> Guizetti,<sup>6</sup> Podvinec and Terplan,<sup>7</sup> and Foot and Olcott<sup>9</sup> thought that possibly infection was an etiologic factor in their cases. These cases, 17 in all, were summarized by Wallgren and will not be reviewed further here except to state that Sherman's case<sup>15</sup> probably does not belong in this category. This case, which showed marked hyperplasia of the reticulo-endothelial system without granulomas, occurred in an 11-day-old infant that had erysipelas, streptococcal septicemia, and jaundice. Klostermeyer's case<sup>17</sup> is regarded as a lymphoid leukemia by several investigators.

Paige<sup>18</sup> presented 2 previously unreported cases of Letterer-Siwe's disease in 1935. Between 1936 and 1940 no additional cases appeared in the literature. A critical review reveals a number of cases, appearing under various titles, which probably belong to this clinicopathologic

syndrome when the accepted criteria are applied. At least one such case, brought to my attention by Paige,<sup>19</sup> was reported as Hodgkin's disease in an infant by Wollstein and McLean<sup>20</sup> in 1926. This case has been reviewed and in retrospect is thought to be a case of Letterer-Siwe's disease. In 1944 Jaffe and Lichtenstein<sup>21</sup> reported a case in an article on eosinophilic granuloma.

Early in 1940 Glanzmann<sup>22</sup> reported a case of Abt-Letterer-Siwe's disease in a 2-year-old female. Some features of this case, namely, osseous defects of the skull, exophthalmos, and presence of lipid in some of the granulomatous lesions, caused him to think that the condition was closely related to the xanthomatosis of Schüller-Christian's disease. Glanzmann regarded the case of Schultz, Wermbter, and Puhl<sup>12</sup> as a transitional form between Letterer-Siwe's and Schüller-Christian's diseases. Wallgren's<sup>13</sup> second case also presented some features of Schüller-Christian's disease (lipid deposit in macrophages and osseous defects of the skull). He postulated that there was an overlapping of the two diseases and that the rapidity of the course of the disease determined the amount of lipid deposited in the macrophages.

In an article on eosinophilic granuloma, Gross and Jacox<sup>23</sup> reported a case of Letterer-Siwe's disease which occurred in a 13-months-old child who died 5 months after onset of symptoms. At autopsy the lungs were thought to resemble those seen in Schüller-Christian's disease, but otherwise the clinico-anatomic complex was similar to that of Letterer-Siwe's disease. Gross and Jacox cited a case reported by Erber<sup>24</sup> in 1931 which they thought belonged in the category of reticulo-endothelial diseases. Siwe<sup>3</sup> did not include this case in his original series nor was it included by Abt and Denenholz<sup>1</sup> or by Wallgren<sup>13</sup> in their series. Lipid was described in some granulomatous lesions in this case and it was probably excluded by the above investigators for that reason.

A number of other cases displaying features of the two diseases and thus thought to represent transitional forms have been reported by Freund and Ripps<sup>25</sup> (1941), Galeotti Flori and Parenti<sup>26</sup> (1937), Freud, Grossman, and Dragutsky<sup>27</sup> (1941), and Sweitzer, Winer, and Cumming<sup>28</sup> (1939). Freund and Ripps reported a case as Schüller-Christian's disease which 14 months before death showed marked proliferation of reticulum cells and formation of syncytial reticulum giant cells in a lymph node taken for biopsy. A very small amount of lipid was present in the reticulum cells, but the diagnosis of Schüller-Christian's disease could not be made until the autopsy material was examined. Characteristic granulomatous lesions with numerous foam cells were found in the lungs and paravertebral fascia, but the lymph nodes

showed changes similar to those seen at biopsy. In their case a mass had appeared in the left groin at the age of 7 weeks but growth and development were normal until the age of 7 months, when pallor and anemia were noted. Later, areas of rarefaction in the skull were demonstrated by roentgenograms. The child died at the age of 2 years following perforation of a gangrenous appendix. The disease in this case was unusually protracted and supports Wallgren's<sup>13</sup> contention that the presence of lipid in the macrophages of granulomatous lesions is related to the duration of the disease. Freund and Rippes cited a similar case reported by Galeotti Flori and Parenti. Biopsy of a lymph node, 1 year before death of a 20-months-old child, showed marked reticulo-endothelial hyperplasia, and the characteristic cells were devoid of lipid. At autopsy, typical lipid granulomas of Schüller-Christian's disease were found.

Freud, Grossman, and Dragutsky<sup>27</sup> reported a case of "acute idiopathic cholesterol granulomatosis," which in many respects was similar to Letterer-Siwe's disease. It occurred in a 7-months-old boy and was rapidly fatal (3 months). The observations at autopsy were similar to described cases of Letterer-Siwe's disease except that there was a large amount of lipid in the involved organs, namely, the thymus, lungs, liver, spleen, and lymph nodes. These investigators accepted their case as supporting Wallgren's<sup>13</sup> tenet that Letterer-Siwe's and Schüller-Christian's diseases are basically the same. The lipid content in this instance was greater than would be expected in a disease existing only 3 months.

Another example of a borderline case is given by Sweitzer, Winer, and Cumming.<sup>28</sup> A 3-year-old boy was well until the age of 2½ years when he complained of pain and weakness in the back and left leg, which limited his activities. Shortly thereafter cutaneous lesions of papular character appeared and anemia developed. Biopsy of the skin showed obliteration of the rete pegs with numerous nonlipid reticulo-endothelial cells in the cutis. Biopsy of the left femur showed many large mononuclear cells, with abundant, finely granular, but only slightly vacuolated cytoplasm and multinucleated giant cells. At autopsy 45 days later many organs were involved with granulomatous lesions which resembled the nodules in the biopsy material.

Merritt and Paige<sup>29</sup> reported a case in which there was marked cutaneous involvement in a child, 3 years of age. Other symptoms had appeared approximately 1½ years earlier. At autopsy there was extensive hyperplasia of the reticulo-endothelial cells without evidence of lipid deposition in the lymph nodes and spleen, while cells of similar

lesions in the thymus, lungs, dura, and bones contained large amounts of lipid and were thought to be typical of Schüller-Christian's disease.

Another case of an intermediate type is that recorded by Grady and Stewart<sup>30</sup> in 1934. The disease occurred in a 3-year-old female who had been ill for approximately 2 years with "running ears." Of particular interest were the lesions in the spleen, some lymph nodes, and portions of the bone marrow, which resembled the granulomas of nonlipid reticulo-endotheliosis. Elsewhere, including the mastoid processes, the lesions were typical of Schüller-Christian's disease.

If the broader concept introduced by Glanzmann<sup>22</sup> and Wallgren<sup>18</sup> is accepted, at least one case (case 1), reported by Lane and Smith<sup>31</sup> in 1939 as "Schüller-Christian disease with cutaneous lesions," can be considered a transitional form bridging the gap between nonlipid (Letterer-Siwe's disease) and lipid reticulo-endotheliosis (Schüller-Christian's disease).

Jaffe and Lichtenstein<sup>21</sup> went further in an attempt to correlate nonlipid reticulo-endotheliosis (Letterer-Siwe's disease) with other diseases involving the reticulo-endothelial system, *i.e.*, idiopathic xanthomatosis (Schüller-Christian's disease) and eosinophilic granuloma. They believed that the different clinico-anatomic observations merely represent varying gradations of severity in the same process—Letterer-Siwe's disease showing the most severe and acute manifestations and being rapidly fatal, with little or no lipid deposition occurring in the reticulo-endothelial cells; Schüller-Christian's disease representing a chronic form of the same disease with a protracted course and the presence of typical foam cells in the lesions which have not been replaced by collagenous connective tissue; and eosinophilic granuloma being the most benign form of the disease with recovery usually occurring. Green and Farber,<sup>2</sup> and Farber<sup>32</sup> had previously suggested that the same basic disorder was responsible for the three diseases.

Several reported cases of reticulo-endotheliosis in adults, variously called aleukemic reticulosis, aleukemic monocytic leukosis, systemic reticulo-endotheliosis, are stated by the authors to resemble reticulo-endotheliosis in infants. Such a case was reported in an adult female, 36 years of age, by Ritchie and Meyer<sup>33</sup> in 1936. Goldzieher and Hornick<sup>34</sup> reported a case in a 75-year-old male and reviewed 22 similar cases appearing in the literature prior to 1931. Not all of these occurred in adults, as he included Akiba's<sup>5</sup> and Letterer's<sup>4</sup> cases. Dameshek's<sup>35</sup> case was in a 51-year-old man. Dameshek stressed the uniformity of the changes in the reticulo-endothelial system irrespective of age and cited cases that occurred from 11 days (Sherman<sup>15</sup>) to 75 years

of age (Goldzieher and Hornick<sup>34</sup>). As yet, there is no convincing evidence that the reticulo-endotheliosis occurring in adults is the same disorder as the infantile form under discussion.

### DISCUSSION

There has been considerable speculation and discussion concerning the etiology of Letterer-Siwe's disease. Siwe<sup>3</sup> divided into two categories the diseases of the reticulo-endothelial system not belonging to the then recognized xanthomatoses (Schüller-Christian's, Gaucher's, and Niemann-Pick's diseases). In the first group he included those cases associated with an acute infection known as infectious reticulo-endotheliosis; in the second group he placed those cases in which no significant infection was present, now known as Letterer-Siwe's disease. Both are regarded as nonlipid reticulo-endotheliosis. Because of the similarity of the histologic changes and the distribution of the characteristic lesions in infectious reticulo-endotheliosis and Letterer-Siwe's disease, Wallgren<sup>13</sup> justifiably challenged the belief that these are separate disease entities. He also presented arguments and evidence for believing that Schüller-Christian's disease is a chronic form of Letterer-Siwe's disease and that, as such, it should be removed from the xanthomatoses.

A critical analysis of the 3 cases reported in this paper indicates that Letterer-Siwe's disease is of noninfectious origin. In each instance, the cutaneous lesions, which I consider a most important and perhaps the earliest manifestation of the syndrome, were present at birth and antedated the fever. There is no reason to believe that an intra-uterine infection was present in any of the cases. Unfortunately, in none was the placenta examined, but there was nothing in the clinical course of the pregnancies to suggest prenatal infection. Case 1 had bilateral otitis media early in the course of the disease, but the possible involvement of the contiguous structures of the ear by reticulo-endothelial granulomas simulating an infectious process must be considered. Glatt,<sup>36</sup> Lichty,<sup>37</sup> and Grady and Stewart<sup>30</sup> described involvement of the middle ears and mastoid processes in Schüller-Christian's disease by granulomas which produced symptoms indistinguishable from chronic otitis media and mastoiditis. The disease was recognizable only after microscopic examination of material removed at operation or autopsy.

If fever is the criterion for infection, infection was present in all previously reported cases as well as in the three presented here, as temperatures above normal were recorded in all at some time during

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the illness. In some the fever was of the Pel-Ebstein type often seen in Hodgkin's disease. An unexplained fever is also often present in leukoses. Since the etiology of these diseases is still unknown, no opinion can be expressed concerning the cause of the fever. Any disease, not primarily infectious, may be accompanied or complicated by concurrent or intercurrent infection, and this may occur also in Letterer-Siwe's disease.

Some anatomic features of these cases tend to support the hypothesis that Letterer-Siwe's and Schüller-Christian's diseases are variants of the same basic disorder. In case 1 there was roentgenographic evidence of skull defects similar to those described in Schüller-Christian's disease (Fig. 2). A number of cases have now been reported in which membranous bones of the skull were involved (Wallgren's<sup>13</sup> case 2, Glanzmann,<sup>22</sup> Abt and Denenholz,<sup>1</sup> and my case 1).

In all cases of the present series variable numbers of cells with vacuolated cytoplasm were found in the typical lesions in one or more sites, although in only one case (case 2) were they at all numerous. Microscopic examination of the spleen or para-aortic lymph nodes in case 2, without a study of additional sections from other organs, might have resulted in a diagnosis of Schüller-Christian's disease. This was also suggested by the appearance of the atypical cells obtained on splenic puncture. It is difficult to reconcile the presence of lipid-containing cells in these cases with Wallgren's hypothesis that the lipid deposit in the granulomatous lesions is a result of chronicity and occurs in the more slowly developing cases. This is especially true of case 3 which occurred in an infant who died at the age of 41 days. Obviously there are factors, other than duration of the disease, which determine lipid deposition. In all cases the thymus showed extensive fibroblastic proliferation and under conditions of chronicity may ultimately have undergone fibrosis and collagenization, features thought to be peculiar to Schüller-Christian's disease.

Another point of anatomic similarity between Letterer-Siwe's and Schüller-Christian's diseases is suggested by the occurrence of cutaneous lesions in approximately one-third of all cases of the latter (Rowland<sup>38</sup>) and in all reported cases of Letterer-Siwe's disease. All variations of the cutaneous eruptions, except the bronze pigmentation and lipid infiltration of the eyelids (xanthoma palpebrarum) described by Chester<sup>39</sup> and by Chester and Kugel<sup>40</sup> as occurring in Schüller-Christian's disease, have been noted in Letterer-Siwe's disease. Petechiae have been noted by all authors. Seborrheic eczema or seborrhea-like eczema was noted by Abt and Denenholz<sup>1</sup> and by Wallgren<sup>13</sup> (case 1).



Papular lesions were described by Wallgren in his case 2, and maculopapular lesions were observed in case 2 of the present series, as were also petechiae and bullous lesions, and a "polymorphous rash" in case 3.

Two or more conditions cannot be considered variants of the same pathologic process just because they are similar anatomically. They must also be similar clinically and have a common cause. All accepted cases of Letterer-Siwe's disease have occurred in infants and young children and have invariably been fatal. An estimated 30 per cent of the cases of Schüller-Christian's disease recover. Generally, Schüller-Christian's disease occurs in children and young adults, which may account for the different clinical outcome in about one-third of the cases. The rapidly fatal cases of Schüller-Christian's disease have occurred in children under 4 years of age. Some of these have shown inconstant amounts of lipid in the granulomatous lesions, and constitute borderline cases. The classical triad of Schüller-Christian's disease depends on the involvement of specific organs or structures by the granulomatous lesions and is encountered too infrequently to be of great value in establishing a diagnosis of that condition. It is not disconcerting that this triad has not been reported in Letterer-Siwe's disease. Glanzmann's <sup>22</sup> case, which had skull defects and unequal bilateral exophthalmos, might conceivably have developed diabetes insipidus if the course of the disease had been of longer duration.

No racial predominance has been noted in Letterer-Siwe's disease. When Rowland <sup>38</sup> reviewed a series of cases of Schüller-Christian's disease in 1928, he concluded that the disease occurred with greater frequency in members of the Jewish race. As increasing numbers of cases have been reported, this racial difference is no longer evident.<sup>41</sup>

The nature of the cells involved in both diseases has interested many investigators. That the principal cells are derived from the so-called reticulo-endothelial system is generally accepted. There is divergent opinion, however, concerning what causes these cells to proliferate. If the proliferation is neoplastic, more pleomorphism should be observed in the proliferating cells. These cells are remarkably uniform from case to case. The cells in the thymus showed the greatest variation. Gross and Jacox <sup>23</sup> reported pleomorphism of cells in the thymus and spleen of their case, suggestive of a sarcoma. The occurrence of mitotic figures in the three cases reported here probably represents rapid proliferation. If mitotic figures were more numerous and if they exhibited abnormal forms, neoplasia would be more tenable. As a rule the mitotic figures were infrequent in regions of reticulo-endothelial proliferation in the thymus, liver, and spleen. They were more common in the foci of re-

ticulo-endothelial cells associated with infection, *i.e.*, in lesions of the skin, tonsils, buccal ulcers, lymphoid tissue of small and large intestine. If, in the future, an infectious agent is implicated, the proliferation of reticulo-endothelial cells must be regarded as a hyperplastic response.

The relationship of eosinophilic granuloma to the two conditions considered above is not so clear although roentgenographically the osseous lesions in the three conditions are indistinguishable. Microscopically, the granulomas of eosinophilic granuloma bear a resemblance to the first two diseases. The most conspicuous constant feature is the presence of large atypical mononuclear cells, many exhibiting phagocytosis. Interspersed among these are variable numbers of eosinophils and giant cells. Foam cells may or may not be present and are usually in direct relation to the duration of the lesions. Clinically, the benign course and the regression and healing of the osseous lesions, either spontaneously or following curettage or radiation therapy, make it difficult to identify eosinophilic granuloma with Letterer-Siwe's and Schüller-Christian's diseases.

Otani and Erhlich <sup>42</sup> and Jaffe and Lichtenstein <sup>21,43</sup> reported lesions of eosinophilic granuloma occurring in long bones and skull. They emphasized the seemingly benign course of the disease. Green and Farber <sup>2</sup> suggested that visceral lesions probably do occur because eosinophils were found in a lymph node biopsy from one of their cases and they advised that a guarded prognosis should be given. Dundon, Williams, and Laipply <sup>44</sup> were of the same opinion. Curtis and Cawley <sup>45</sup> have reported a case of eosinophilic granuloma of bone with cutaneous manifestations of the disease verified by biopsy. Weinstein, Francis, and Sproffkin <sup>46</sup> recently have reported a case with multiple osseous lesions of eosinophilic granulomatous type. The case had roentgenographic evidence of pulmonary infiltration similar to that seen in Schüller-Christian's disease. The patient recovered from the osseous and pulmonary lesions.

Jaffe and Lichtenstein <sup>21</sup> stated that eosinophils are found only in the osseous lesions of Letterer-Siwe's disease. In all three of my cases, eosinophils were found in visceral as well as skeletal granulomas. Eosinophils also occur in the granulomatous lesions of Schüller-Christian's disease. Gross and Jacox, <sup>23</sup> in a review of 84 cases of Schüller-Christian's disease, found that eosinophils occurred in variable numbers in 29 cases. Fraser's <sup>47</sup> article on skeletal xanthomatosis contains several colored drawings of typical granulomas of Schüller-Christian's disease, which are indistinguishable from the lesions of eosinophilic granuloma. Thannhauser <sup>48,49</sup> contended that eosinophilic granuloma of the bone is

the "monosymptomatic form of a well known systemic granulomatous disorder [Schüller-Christian's disease] in which histiocytes, eosinophils and xanthoma cells are observed in the lesion at different phases." Jaffe and Lichtenstein<sup>50</sup> believed that it is necessary to make a clinical distinction between eosinophilic granuloma of bone and Schüller-Christian's and Letterer-Siwe's diseases even though morphologically they apparently represent different phases of the same basic disorder.

### CONCLUSIONS

Including the 3 cases reported in this paper, the number of cases which meet the established criteria for Letterer-Siwe's disease does not exceed 24.

This study has not disclosed the cause of Letterer-Siwe's disease, but it does offer evidence that the disease is not initiated by infection. In all cases, the cutaneous lesions preceded by 2 to several weeks the manifestations of infection, *i.e.*, sore throat, otitis media, diarrhea, and fever. Infection must therefore be considered secondary or intercurrent, and not causative.

That Letterer-Siwe's and Schüller-Christian's diseases represent different manifestations of the same basic disorder of the reticulo-endothelial system seems probable from the number of borderline or transitional cases which have been reported. Twelve such cases were found in a review of the literature. That such a relationship does exist between these two diseases is accepted by Green and Farber,<sup>2</sup> Jaffe and Lichtenstein,<sup>50</sup> Mallory,<sup>51</sup> and Letterer,<sup>52</sup> as well as by Wallgren.<sup>13</sup>

That a possible relationship exists between the above two conditions and eosinophilic granuloma has been suggested by the same investigators. Thannhauser<sup>48,49</sup> believed that eosinophilic granuloma is actually a phase of the disorder known as Schüller-Christian's disease and as such should not be considered as a separate clinical entity.

Regardless of the morphologic similarity among the three diseases, the clinical course of eosinophilic granuloma varies so greatly from that of the other two conditions that a sharp clinical distinction between these disorders of the reticulo-endothelial system is justified until a common etiologic factor has been demonstrated for all.

I wish to acknowledge my indebtedness to Dr. C. F. McKhann of Babies and Childrens Hospital, University Hospital, Cleveland, Ohio, who kindly gave permission to use the clinical records of the three cases presented in this paper.

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[ Illustrations follow ]

## DESCRIPTION OF PLATES

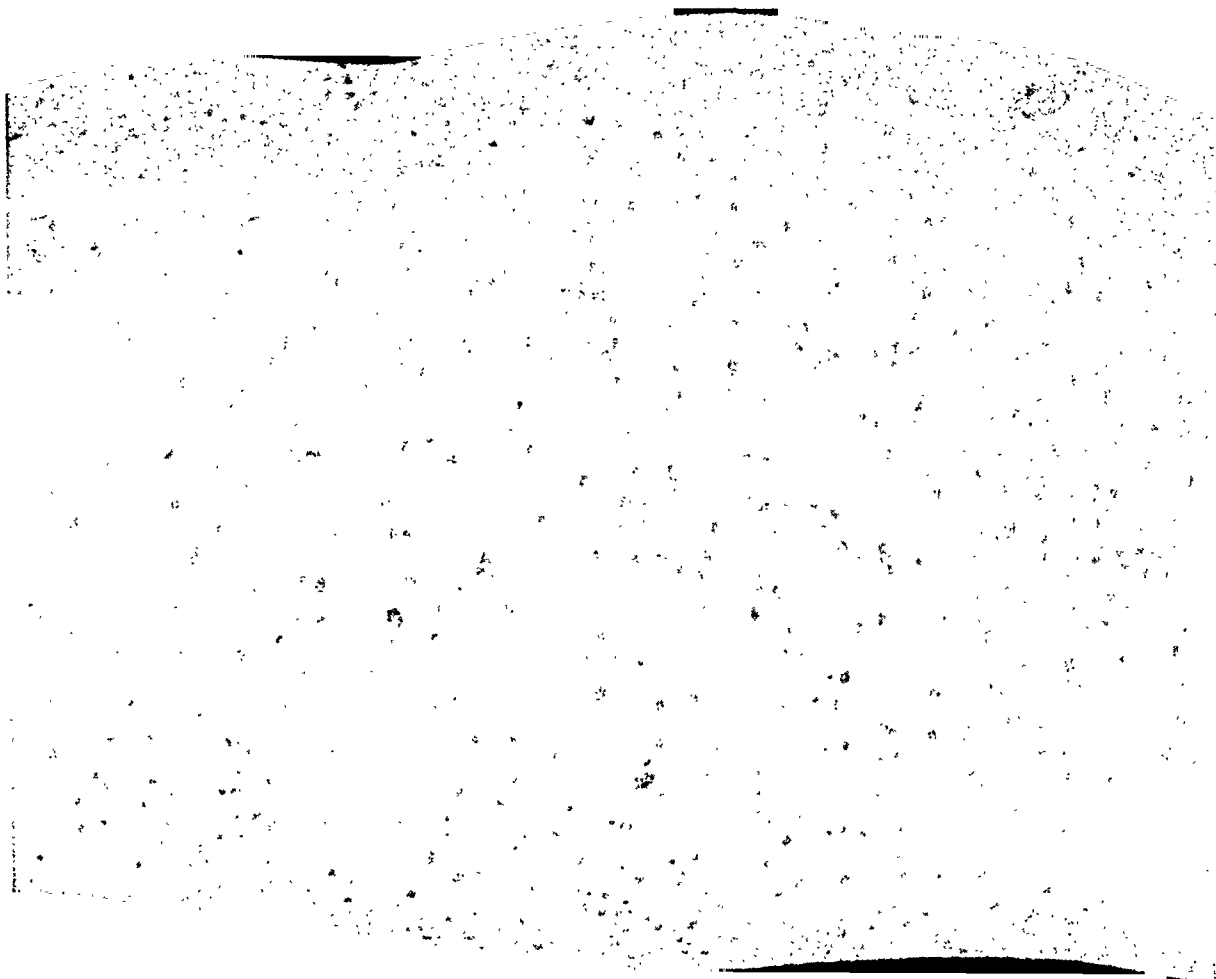
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### PLATE 7

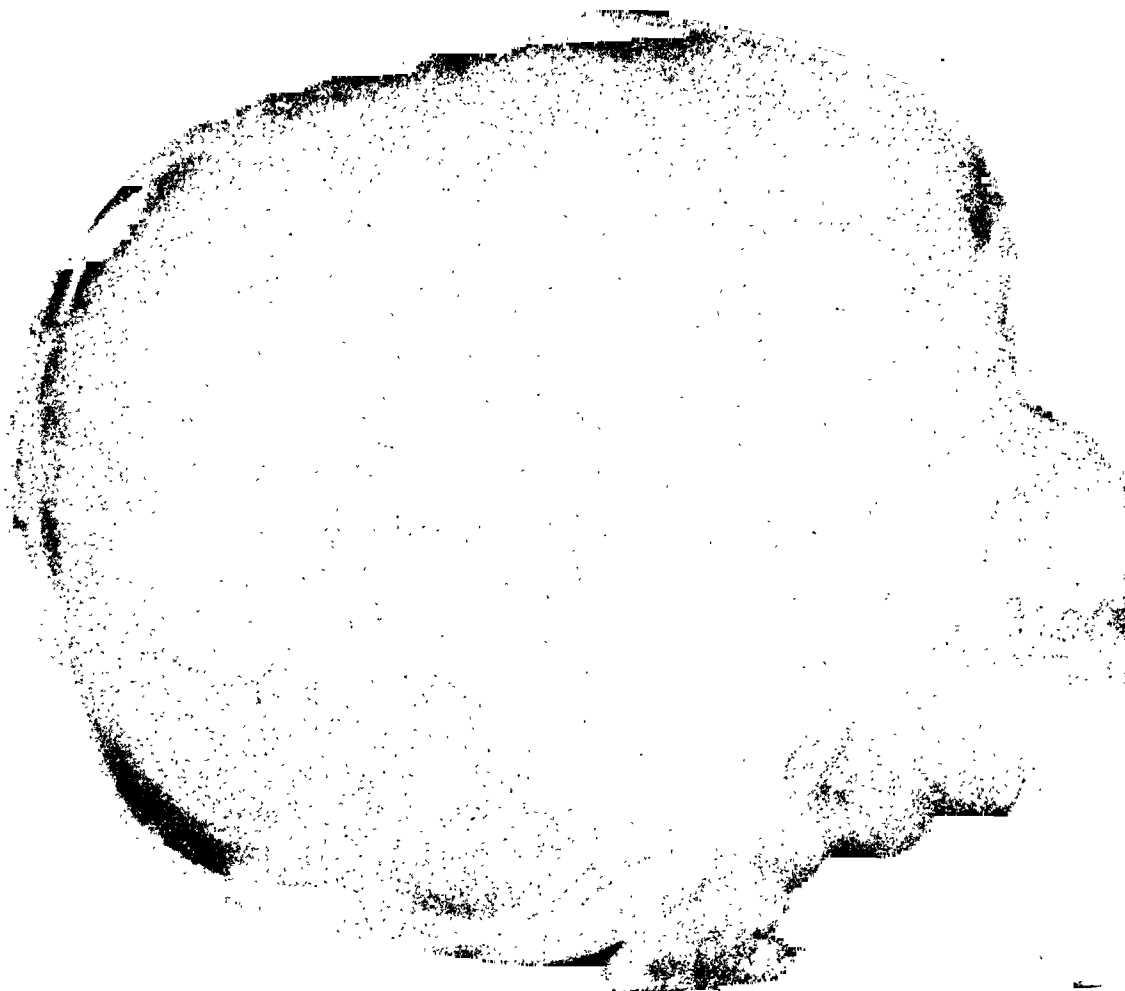
FIG. 1. Case 1. Lateral view of body showing numerous cutaneous lesions confined chiefly to the torso. Many have hemorrhagic centers.

FIG. 2. Case 1. Roentgenogram of skull showing osseous defects in the right parietal and occipital bones.

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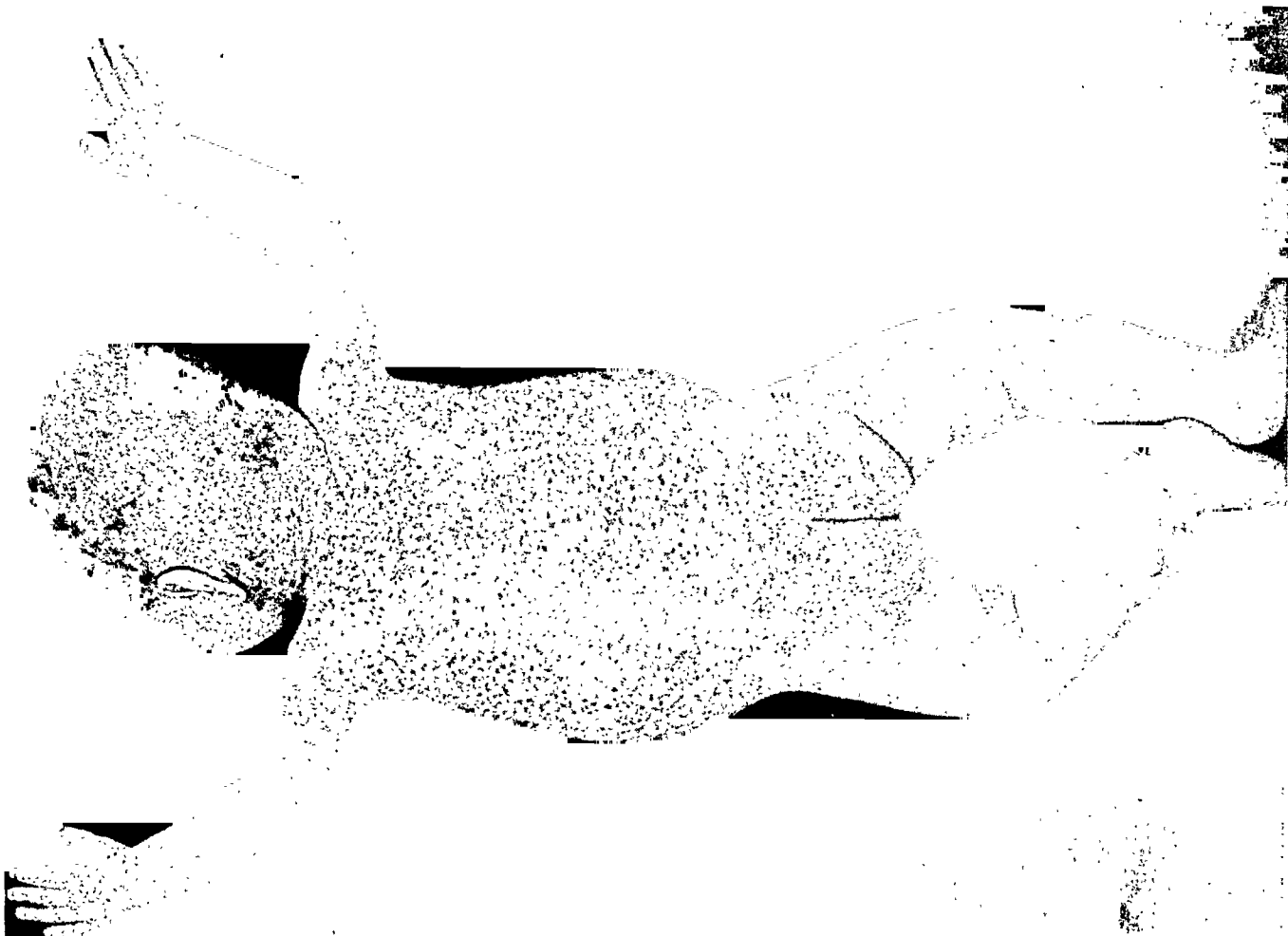




### PLATE 8

- FIG. 3. Case 2. Dorsal view of body to show diffuse involvement of trunk and scalp with cutaneous lesions. The extremities were spared in this case. The lesions were crusted maculopapules and petechiae. The increased transverse diameter of the body below the level of the ribs is due to hepatic and splenic enlargement.
- FIG. 4. Case 1. Cutaneous lesion, showing a collection of atypical mononuclear cells in the corium and atrophy and parakeratosis of the overlying epithelium. There is a minute abscess in the epithelium. For comparison with the photomicrograph of the cutaneous lesion in case 2 (Fig. 5). Hematoxylin and eosin stain.  $\times 186$ .

3



4

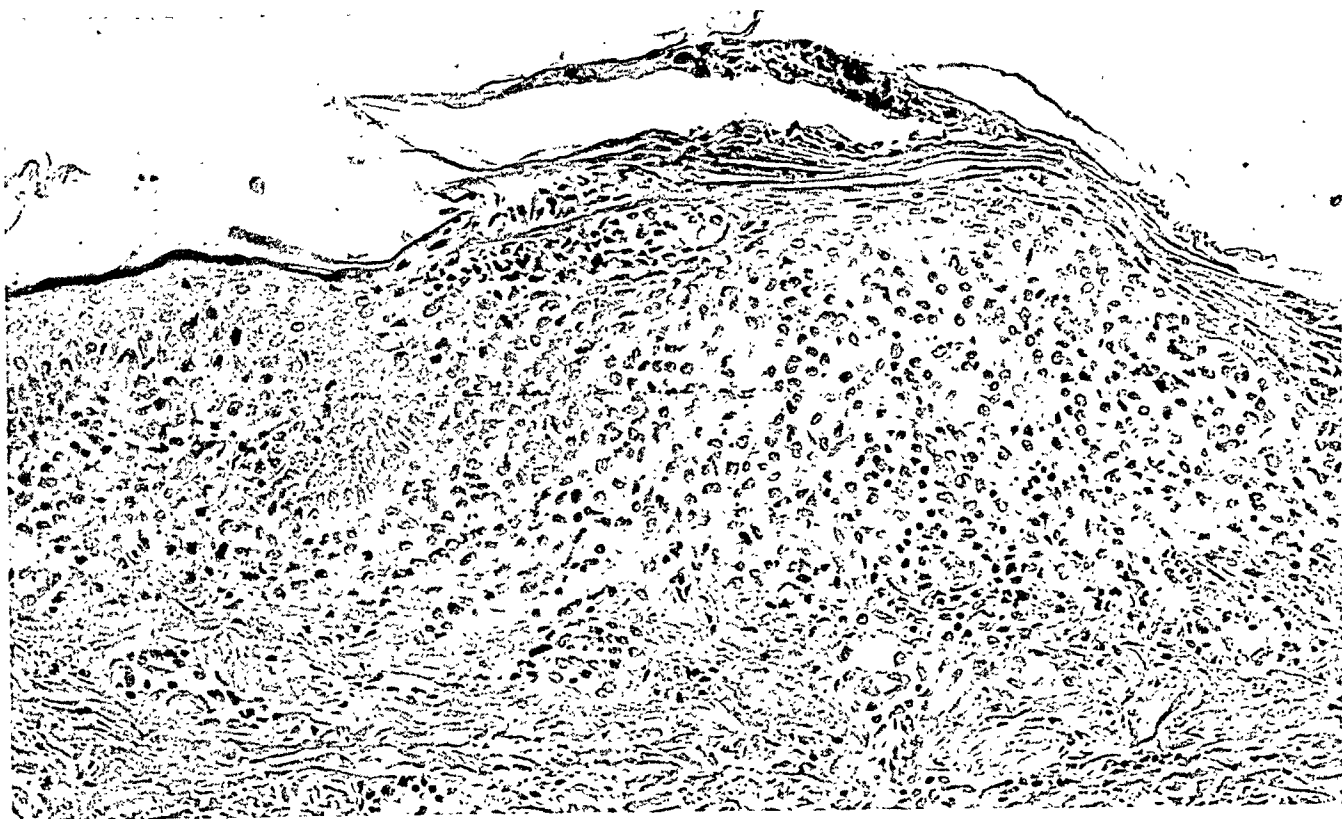
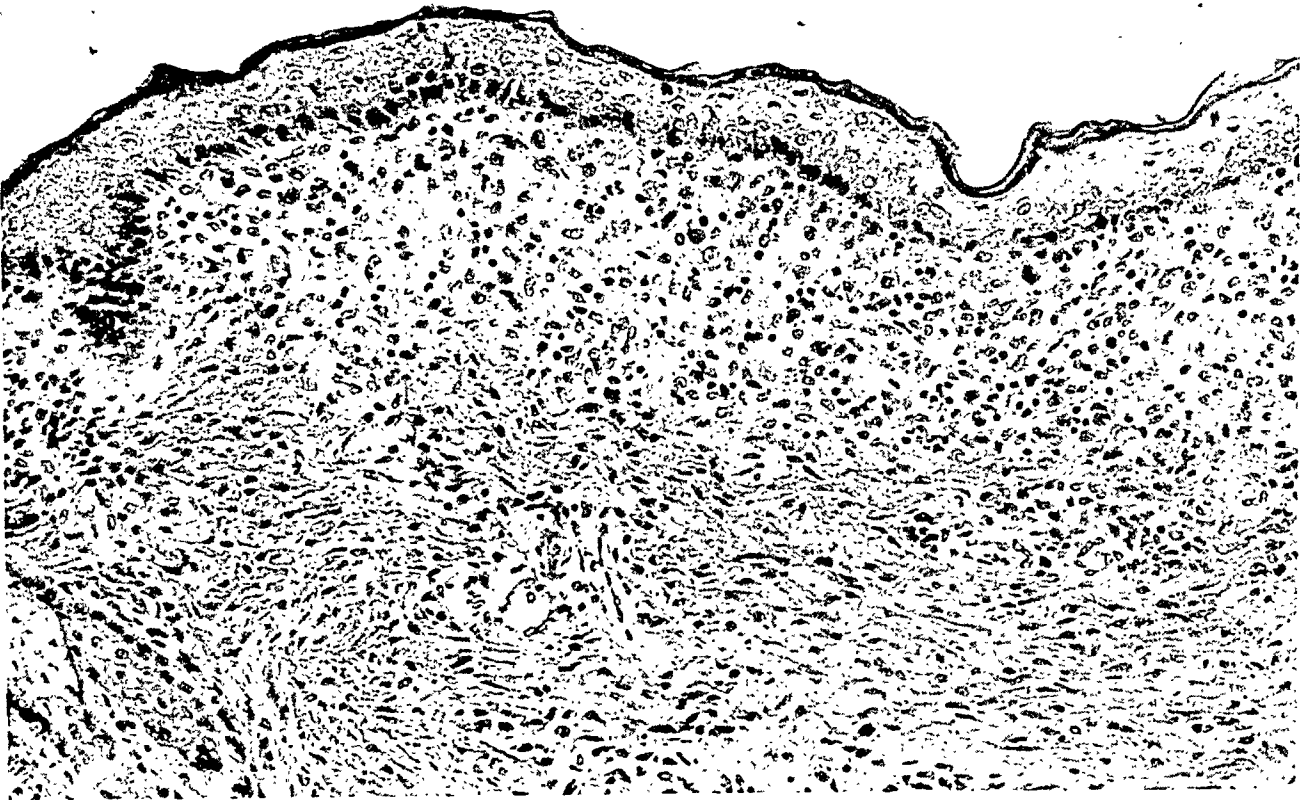


PLATE 9

FIG. 5. Case 2. Cutaneous lesion. This photomicrograph shows marked similarity to Figure 4. Hematoxylin and eosin stain.  $\times 186$ .

FIG. 6. Case 1. Thymus showing numerous giant cells; one in the lower center field shows marked phagocytic activity. Of note are the characteristic mononuclear cells with vesicular nuclei and the irregular outline of the giant cells and mononuclear reticulo-endothelial cells. Hematoxylin and eosin stain.  $\times 342$ .

5



6

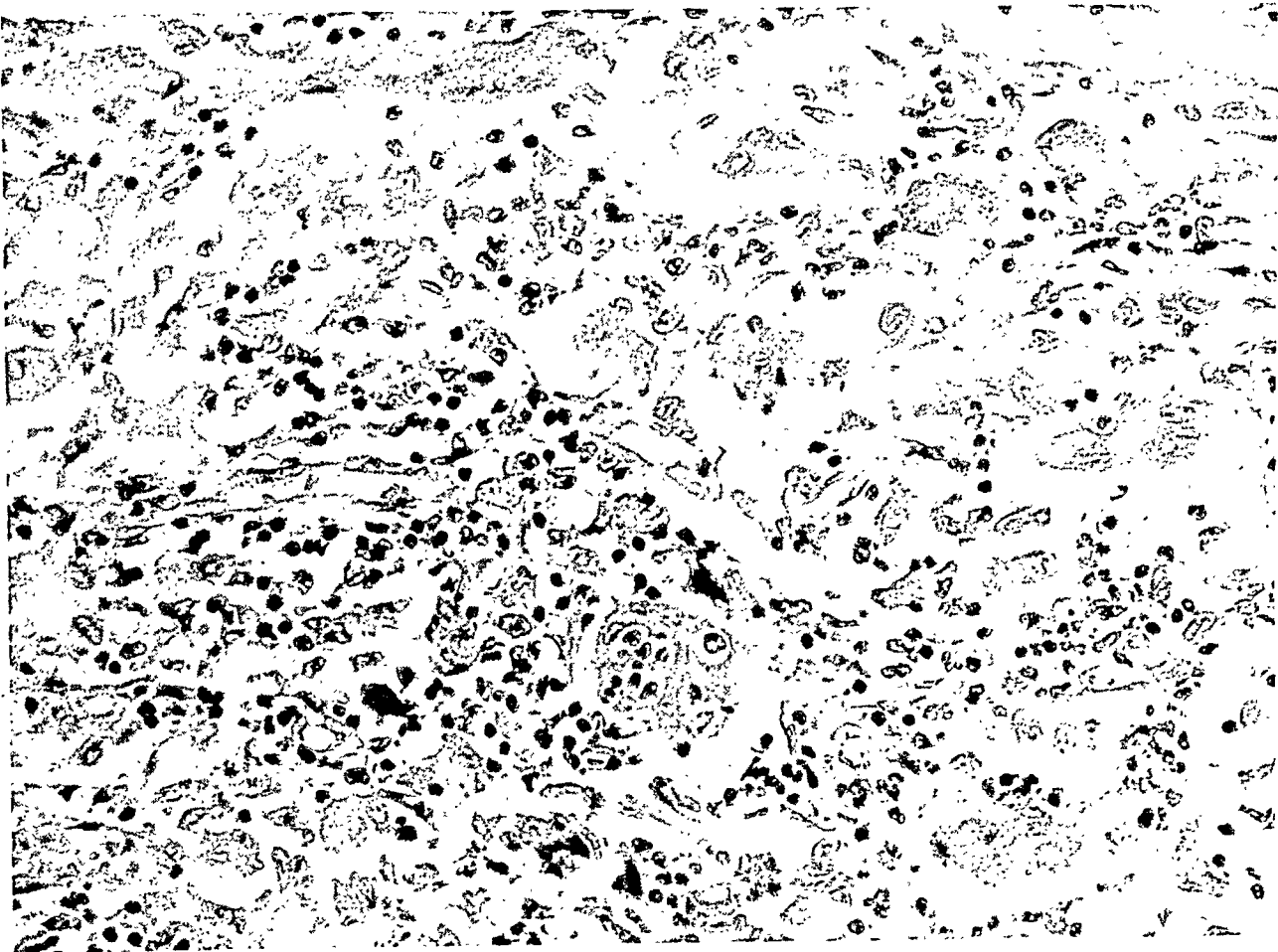
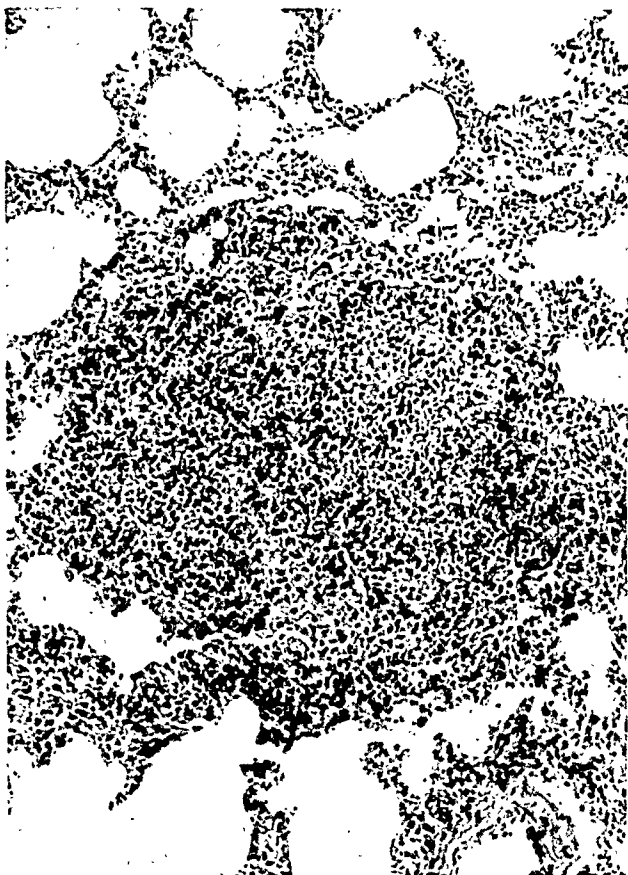


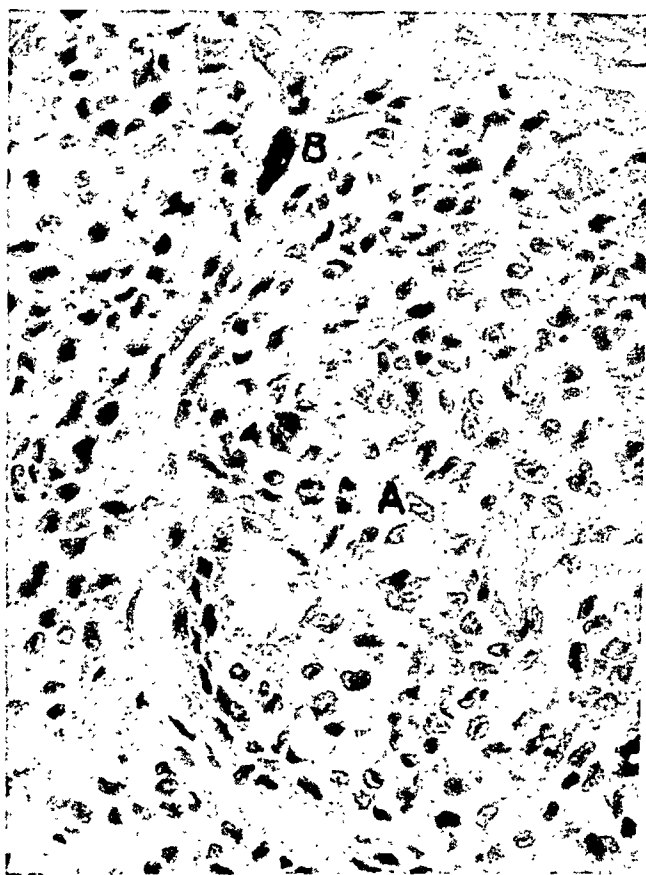
PLATE 10

- FIG. 7. Case 3. Lung showing a characteristic nodule of reticulo-endothelial cells. Similar cells are present in alveolar septa. Hematoxylin and eosin stain.  $\times 80$ .
- FIG. 8. Case 1. The subepithelial infiltrate in the base of the gingival ulcer. Several mitotic figures are present at A; fibroblastic proliferation and bizarre-shaped nuclear form at B. Hematoxylin and eosin stain.  $\times 342$ .
- FIG. 9. Case 1. Peyer's patch of the ileum completely replaced by large mononuclear cells. The small, dark cells are lymphocytes. Hematoxylin and eosin stain.  $\times 186$ .

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8



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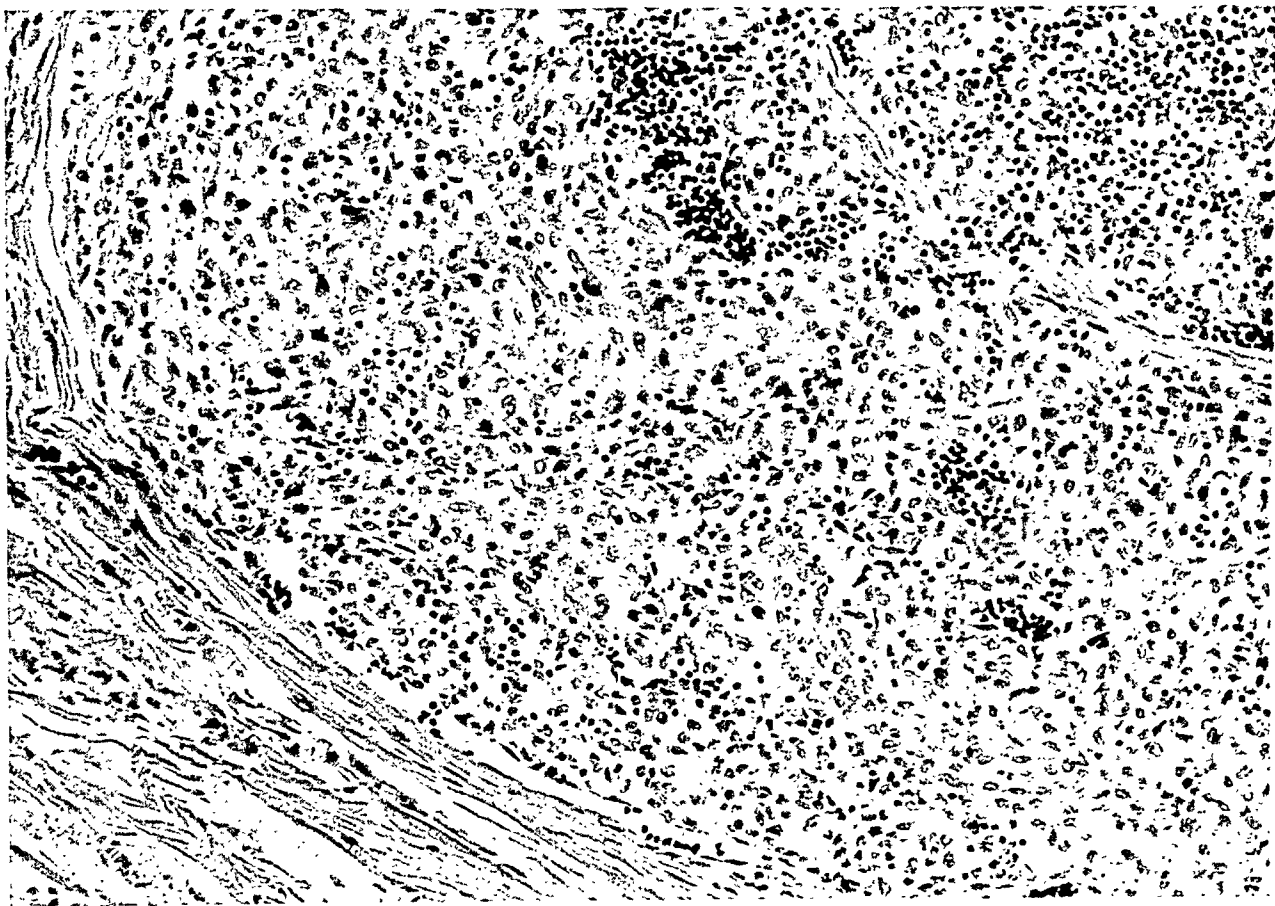
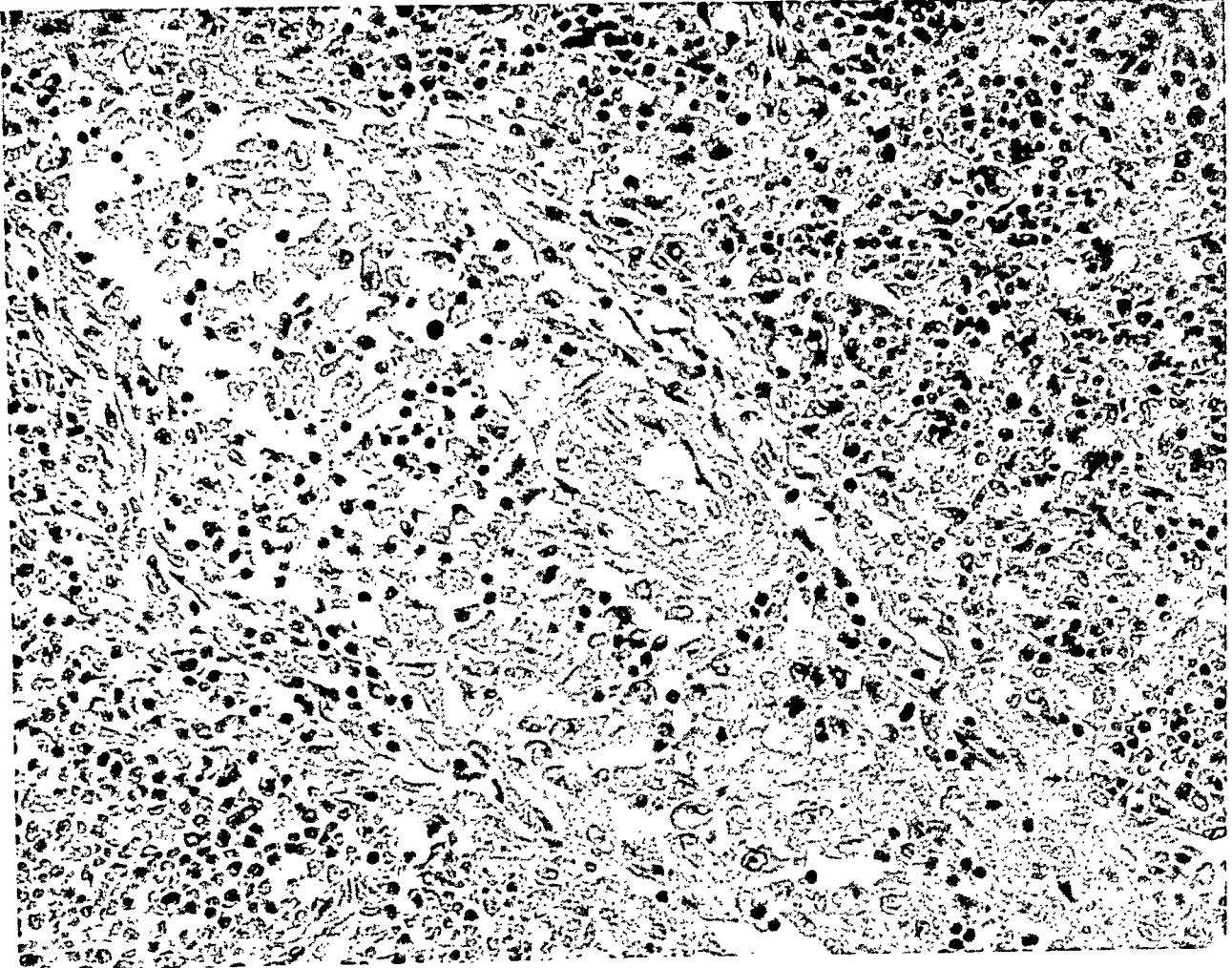


PLATE II

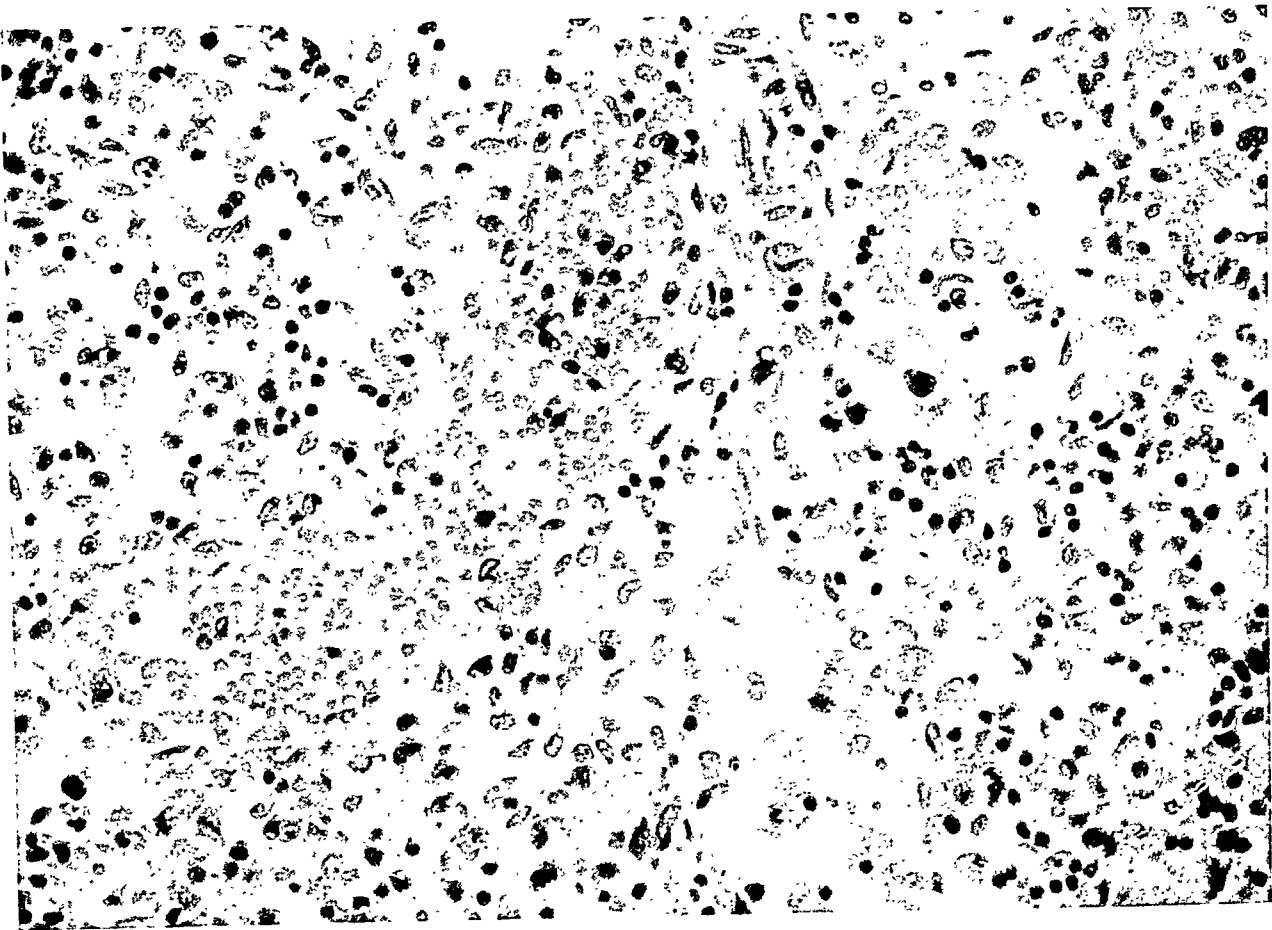
FIG. 10. Case 1. A nodule of mononuclear cells near a central arteriole in the spleen. There is fibroblastic proliferation surrounding the nodule. A variety of cells may be seen in the splenic pulp. Hematoxylin and eosin stain.  $\times 342$ .

FIG. 11. Case 2. Lymph node showing collections of large mononuclear cells, some of which have vacuolated cytoplasm, others contain phagocytized cells. Hematoxylin and eosin stain.  $\times 342$ .

10



11







# THE NATURE OF THE DOUBLE-CONTOURED AND STRATIFIED INTRACELLULAR BODIES IN SARCOIDOSIS (BOECK-SCHAUMANN)\*

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The occurrence of double-contoured, stratified, and in some cases calcified, bodies in the pathologically altered lymph nodes and tonsils of patients suffering from sarcoidosis was first observed by Schaumann,<sup>1</sup> who described them in 1917 and found them in particular in tonsils and lymph nodes with a markedly tuberculoid structure. In 1941 Schaumann<sup>2</sup> again described these structures in detail and stated that, as a rule, they are contained in giant cells in which some of them may assume such large size that they occupy the entire giant cell, possibly causing it to burst. In many cases the corpuscles lie in the intercellular spaces in relation to more or less distinct remnants of the giant cells. Schaumann was able to demonstrate the occurrence of such stratified bodies in practically all organs with sarcoidosis, including lungs, spleen, and bone marrow.

Tillgren<sup>3</sup> described them in the granulomata in the pituitary gland in a case of Boeck's sarcoid with diabetes insipidus. The bodies also have been observed by Bergstrand,<sup>4,5</sup> among others, who pointed out that their occurrence supports the diagnosis of Boeck's sarcoid, and by Lindau who, in one of Lemming's cases,<sup>6</sup> described them as concentric, stratified, round, or irregularly ramified bodies which were not calcified or acid-fast and were not stained by Gram's method. In this connection, mention should be made of Weller's observation<sup>15</sup> (1922) of laminated concretions in 3 of 142 cases of "tonsillar tuberculosis, diagnosed histologically." At that time he probably included cases of sarcoidosis.

The intracellular "asteroid bodies" described by Friedman<sup>7</sup> and others in sarcoidosis of the spleen must doubtless be considered as being essentially of the same nature.

## PREVIOUS VIEWS OF THE NATURE OF STRATIFIED BODIES IN BOECK'S SARCOIDOSIS

Most of the authors who have previously examined these structures have considered them as being products of degeneration of elastic fibrils<sup>4</sup> impregnated with calcium and iron salts, whereas Schaumann<sup>2</sup> has stated that the possible occurrence of elastic threads in these structures is not of decisive importance as to their genesis. The presence of connective tissue fibrils, hairs, and tubercle bacilli in these bodies also

\* Received for publication, January 26, 1948.

is said to have been ascertained. Moreover, Schaumann<sup>2</sup> referred to Metchnikoff's production of quite similar bodies by the inoculation of tubercle bacilli into the Algerian gerbil. In accordance with the marked resistance of this rodent to tuberculosis, the tubercles of the inoculated gerbil as a rule show no signs of necrosis, a fact which might correspond to the findings in Boeck's sarcoid. Considering Metchnikoff's observations and the view of Boeck's sarcoid as being of tuberculous nature, Schaumann<sup>2</sup> advanced the opinion that tubercle bacilli can be transformed into such bodies. He also believed that the bodies may result from the deposition around elastic fibrils, connective tissue fibrils, or a hair, of substances resulting from disintegrated tubercle bacilli in their acid-fast or nonacid-fast form.

### OBSERVATIONS

In a recent publication,<sup>8</sup> as the basis of the morphogenesis of the lesions in Boeck's sarcoid and in other conditions, I described an (allergic) *hyperglobulinosis in the reticulo-endothelial system passing on to hyalinosis (or paramyloidosis)*, representing a typical development, by stages, of the sarcoid lesions and presumably the essential change in their structure. In addition it was demonstrated<sup>8,9</sup> that pathogenetically analogous reactions underlie certain morphologic lesions, *inter alia*, lupus erythematosus disseminatus and atypical and experimental amyloidosis. These findings afford an explanation of the nature of the hyperglobulinemia in these states, and in Boeck's sarcoidosis they explain the development by stages of the granulomata and the extragranulomatous changes from reticulosis through (hyperglobulinotic) precipitation to hyalinosis (paramyloidosis) in and around the granulomata.

These findings also illustrate the absence of necrosis, the intensive red staining of the granulomata by Unna's staining method, and the marked accumulation of plasma cells observed in connection with the perigranulomatous precipitates, marked hyalinosis, and paramyloidosis.

I have considered<sup>8</sup> the described elementary reaction in Boeck's sarcoidosis and the other states mentioned, all of which are accompanied at certain stages by hyperglobulinemia as a characteristic symptom, to be a *morphologic immunity reaction*, which shows the importance of plasma cells and of other reticulo-endothelial cells in hyalinosis and atypical amyloidosis in a number of different states. In the case of Boeck's sarcoidosis, such a view is in complete conformity with the general perception of this disorder as a condition with high immunity (positive anergy).

In my case 2 of Boeck's sarcoidosis<sup>8</sup> in a woman, 28 years of age,

with no bacteria in the expectoration and with a negative Mantoux test on repeated examinations, roentgenologic examination and autopsy showed the picture of a bilateral cavernous pulmonary tuberculosis (with no tubercle bacilli either on inoculation into guinea-pigs or on cultivation), combined with typical sarcoidosis in the spleen, lymph glands, liver, and lungs. The pulmonary changes, as well as the fact that the patient's brother had died of tuberculosis, favored tuberculosis as the cause in this case. On the other hand, the highly marked immunity manifested itself through widespread lesions of sarcoidosis (passing on to very marked paramyloidosis) in the reticulo-endothelial system, in particular in the spleen and in the lymph glands, and through the absence of bacteria on inoculation and cultivation from the cavernous foci.

In general, the typical granulomata of Boeck in the spleen and lymph nodes were subject to hyaline or paramyloid transformation, wholly or in part. Many of the granulomata in the spleen, lymph nodes, and lungs were found to contain stratified bodies in accordance with the descriptions given by Schaumann<sup>2</sup> and Skavlem and Ritterhoff.<sup>10</sup> In some instances they were ring-shaped, but usually they were concentrically stratified, double-contoured, almost everywhere situated in giant cells, filling them out wholly or in part (Fig. 1), and often several occurred within the same granuloma (Fig. 2). The bodies stained deep red by Unna's method, most frequently a still deeper red than that of the granulomata and the protoplasm of the plasma cells. This is considered indicative of a content of ribose nucleotide, which is regarded as characteristic of the protein formation in cells and presumably gives rise to their basophilia. In the centers of some of the bodies, there could be demonstrated oblong structures, resembling foreign bodies, surrounded by concentric stratified precipitated material. A conspicuous feature in this case and in other cases of widespread sarcoidosis in the same phase was the occurrence of numerous foreign body giant cells in which the nuclei were placed quite irregularly (Fig. 3) and which resembled osteoclasts. Generally, comparatively few giant cells are seen in this disease. By means of Unna's and Masson's staining methods it was possible in several areas corresponding to the presumably earlier stages of the precipitation to demonstrate intensely red-staining crystalline precipitates or confluent hyaline droplets in direct relation to protoplasm of the reticulum cells and also of a deep red color (Fig. 4). This confirmed the impression that these were products from an intracellular globulin precipitate. Impregnation with iron and calcium may take place later on.

On the basis of these and my previous findings,<sup>8</sup> I believe that it has

been rendered highly probable that the stratified bodies in Boeck's sarcoidosis are due to biochemical (partly crystalline) precipitates of the nature of a globulin in the cytoplasm, where they behave like foreign bodies. The supposition that they are a product of transformed tubercle bacilli seems to me to be improbable. The nature of the change as a *hyperglobulinotic* reaction, expressing an especially high degree of immunity, fully explains the occurrence of stratified bodies in Metchnikoff's inoculation experiments referred to above and in the pathologically changed tissue in Boeck's sarcoidosis. The occurrence of "transformed tubercle bacilli," as supposed by Schaumann,<sup>2</sup> may be considered theoretically for certain organs such as the lungs, whereas the lesions in sarcoidosis with regard to localization (spleen, lymph nodes, posterior lobe of pituitary body) and structure (extragranulomatous changes) appear as a reticulosis which cannot presuppose the occurrence of tubercle bacilli in these organs. (See also Lemming's<sup>11</sup> production of typical lesions of Boeck at the site of injection after the administration of B.C.G. vaccine to a patient with histologically verified Boeck's sarcoidosis and persisting negative Mantoux's reaction.)

The view advanced here does not preclude, however, that an inoculation with tubercle bacilli, as in Metchnikoff's experiment, or the occurrence of tubercle bacilli in certain tissues, *e.g.*, in the lungs, may give rise to the same morphologic immunity reaction.

Concentric stratified globulinoid precipitates which, as far as structure, similarity of color, and a reaction like that to foreign bodies are concerned, bear a close resemblance to the stratified bodies in Boeck's sarcoidosis have been demonstrated also in the kidneys in plasma cell myeloma (Apitz<sup>12</sup>), an essentially different disorder which, however, in common with sarcoidosis displays hyperglobulinosis and hyperglobulinemia as products of plasma (or other reticulo-endothelial) cells with a tendency to paramyloid precipitation.

Lastly, the monstrous forms of foreign body giant cells containing intracellular, clumpy precipitates in cases of chronic Gaucher's disease<sup>13</sup> and the giant cells which can be observed in atypical amyloidosis after immunization<sup>14</sup> are other instances of a giant cell reaction caused by a biochemical intracellular precipitation in reticulum cells.

#### SUMMARY

On the basis of the view of Boeck's sarcoidosis advanced in my previous publications and interpreting the morphologic lesions in this disorder as representing a serologic hyaline (paramyloid) precipitation, having as its starting point a globulin-precipitate (allergic hyperglobu-

linosis) in the reticulo-endothelial system, the "peculiar corpuscles" described by Schaumann as being present in the tissue of sarcoidosis (Boeck-Schaumann) are considered representative of a hyperglobulinotic (hyaline or crystalline) precipitate in the cytoplasm of the reticulo-endothelial cells.

The staining reactions of the precipitates with Unna's and Masson's methods are identical with those of the pre-hyaline precipitates and of the cytoplasm in plasma cells and the reticulum cells of the granulomata, and must be considered indicative of a content of ribose nucleotide.

In the proper stages of sarcoidosis, the crystalline precipitates give rise to the occurrence of numerous foreign body giant cells.

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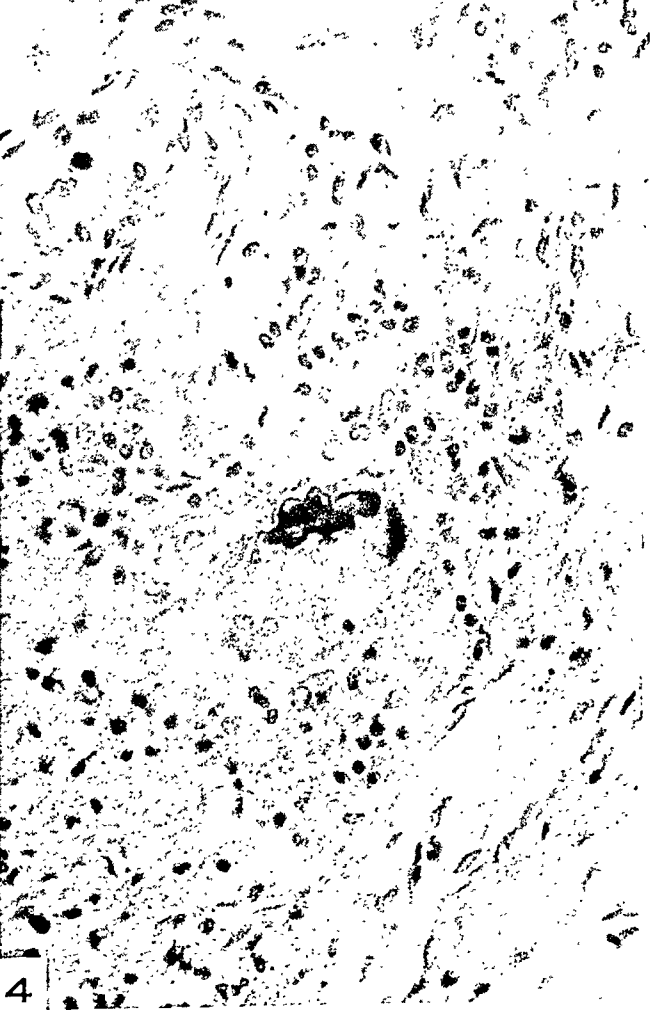
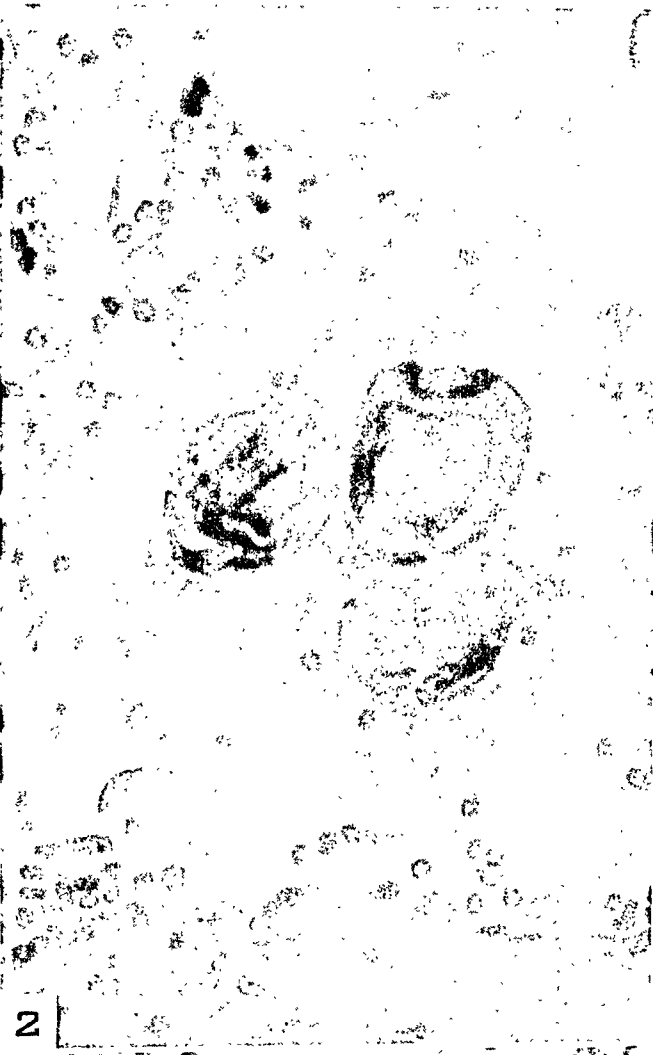
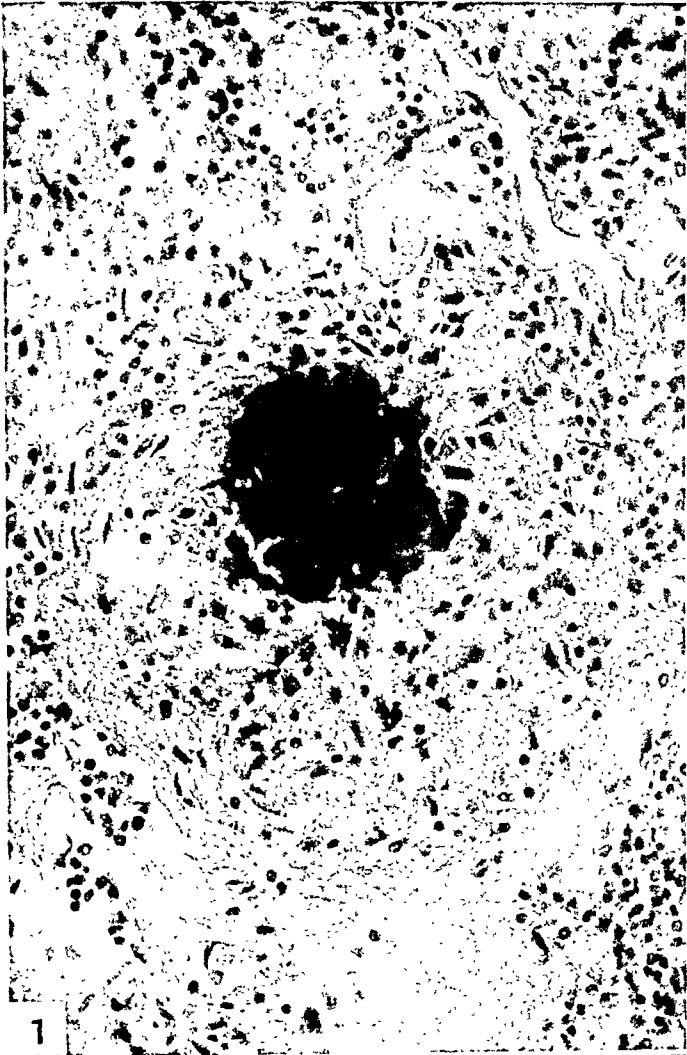
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## DESCRIPTION OF PLATE

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### PLATE 12

- FIG. 1. Stratified body completely filling a giant cell in a granuloma in a lymph node in sarcoidosis. Unna's stain.  $\times 270$ .
- FIG. 2. Three stratified bodies in the same granuloma in sarcoidosis. Unna's stain.  $\times 370$ .
- FIG. 3. Foreign body reaction around a stratified body. Unna's stain.  $\times 370$ .
- FIG. 4. Precipitation of confluent hyaline droplets in direct relation to disintegrated cytoplasm of reticulum cells. Unna's stain.  $\times 370$ .



Teilum

Intracellular Bodies in Sarcoidosis





# THE HISTOGENESIS OF CLEAR CELL PAPILLARY CARCINOMA OF THE SKIN \*

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Clear cell papillary carcinoma, so designated because of its predominating clear cuboidal-shaped cells arranged in papillary manner, is a variety of basal cell carcinoma. As far as I am aware, this tumor rarely has been discussed in the literature and its origin has not been fully investigated. The so-called simple adenoid carcinoma, believed by Ewing<sup>1</sup> to originate in hair follicles and in which the tumor exhibits papillary or alveolar masses lined by cuboidal or low-cylindric and occasionally squamous cells, seems to be morphologically similar to this tumor. The slowly growing "cubo-celled carcinomata" mentioned by Krompecher,<sup>2</sup> Bloodgood,<sup>3</sup> and Hazen<sup>4</sup> also have clinical and histologic similarities.

Clear cell papillary carcinoma is comparatively rare. Among 700 cases of carcinoma of the skin (including those arising from the penis and vulva) examined in the surgical pathologic laboratory of the Department of Pathology of the Peiping Union Medical College, only 4 such tumors have been seen. The purpose of this report is to present the clinical and histologic features of these 4 tumors and to suggest that they were derived from hair follicles.

## REPORT OF CASES

### Case 1

A student, 20 years of age, came to the surgical out-patient department of the Peiping Union Medical College Hospital on January 18, 1941, because of a firm, bean-sized nodule in the axillary region. The exact duration of the tumor was not known. It was slowly growing, movable, and cystic. It was excised under local anesthesia. There was no evidence of recurrence 6 months after the operation.

*Clinical Diagnosis.* Sebaceous cyst or hemangioma.

### Pathologic Examination

The specimen consisted of a tumor nodule, well encapsulated, 1.5 cm. in diameter, covered on one side by a piece of skin. Section revealed grayish, homogeneous tissue containing many small cystic spaces filled with blood clot. Microscopically, the tumor was made up of papillary or alveolar masses composed chiefly of clear cuboidal and low-cylindric cells with a varying amount of connective tissue stroma (Fig. 3). The cells near the base of the papillary growth were low-cylindric, showing palisade formation, while the more superficial cells were polyhedral and irregularly arranged and the cytoplasm was mostly clear or scanty.

\* Received for publication, December 12, 1947.

The clear cells had prominent borders and rather small pyknotic nuclei. The cylindric cells had finely granular cytoplasm, although they were often quite clear. Mitotic figures were seen only occasionally among the cylindric cells. The most superficial cells (near the skin) were prickly cells showing a tendency to cornification but no definite pearl formation. The papillary masses were closely packed, but occasionally there were crevices or clear spaces. In between them was either thin fibrous or dense hyalinized tissue. Scattered among the clear cells were small, circular, glassy epithelial products (hair shafts) surrounded by flattened or concentrically arranged tumor cells (Fig. 10). These structures stained reddish by Masson's method and yellowish with van Gieson's stain. When Foot's silver method was used they were blackish in varying degrees of intensity, being lighter in the center and deeper at the periphery. With this technic, occasionally a deeply stained, membrane-like object could be seen surrounding the glassy "hair shaft." Similar structures were seen in normal hair follicles with this method. Best's carmine staining of the neoplastic tissue revealed abundant glycogen in the cytoplasm of both clear and nonclear cells.

*Diagnosis.* Slowly growing, clear cell papillary carcinoma of hair-follicular origin.

### Case 2

A woman student, 21 years of age, was seen in Douw Hospital, Peiping, in May, 1938, complaining of a subcutaneous growth on the left arm of about 2 years' duration. It had grown slowly and was symptomless. At the time of her visit the tumor had reached a diameter of about 1.5 cm.

### Pathologic Examination

The specimen consisted of a roundish tumor mass measuring 1.5 by 1.0 by 0.8 cm. The cut surface revealed homogeneous whitish tissue with irregular blood-stained spaces. Microscopically, the tumor presented a picture identical with that of case 1 (Fig. 4).

*Diagnosis.* Slowly growing, clear cell papillary carcinoma arising from a hair follicle.

### Case 3

A housewife, 48 years of age, was admitted to the William Porter Hospital, Techow, Shantung, on July 10, 1940, because of a slowly growing tumor of the right thigh of 20 years' duration. When first noticed, the tumor was about the size of a bean, movable beneath the skin. Three years before admission, it had been incised by a country doctor and grew rapidly thereafter. At the time of hospitalization it was about 12 cm. in diameter.

### Pathologic Examination

The specimen consisted of a rectangular block of tissue measuring 2.0 by 1.6 by 0.8 cm. One edge was covered by skin. The cut surfaces showed a grayish tissue containing cystic spaces (Fig. 2). Micro-

scopically, the picture was much the same as that seen in the previous cases (Fig. 5). Scattered among the clear cells were occasional, large, foamy cells containing fat globules, as shown when stained by sudan III. In many places the basal cells showed squamous changes and were heavily loaded with glycogen granules.

*Diagnosis.* Slowly growing, clear cell papillary carcinoma arising from a hair follicle.

#### Case 4

A farmer, 48 years of age, was seen in the William Porter Hospital, Tschow, Shantung, in November, 1940, because of a small mass of about 2 years' duration on his right upper eyelid. At first the tumor had grown slowly, but had assumed a more rapid course following needling and the use of a Chinese plaster, 6 months after its appearance. For the past few months the tumor had bled frequently and there was a continuous discharge of pus and yellowish fluid.

On admission, the general physical and laboratory findings were not remarkable. Locally, an ulcerated tumor the size of a hen's egg was seen to rest upon the arcus infra-orbitalis. It was hard, immobile, and bled easily when touched. The right pre-auricular region showed a small, freely movable lymph node. The tumor was excised and the eyeball enucleated.

#### Pathologic Examination

The specimen consisted of a tumor measuring 3.5 by 3.0 by 5.0 cm., an eyeball, its attached muscles, and a small lymph node 0.8 cm. in diameter. The covering skin was adherent and showed ulceration and necrosis at its lower margin. Section revealed grayish neoplastic tissue interlaced by thin, glistening stroma (Fig. 1). The eyeball and the lymph node were free from tumor. Microscopically, the tumor showed clear cells, polyhedral prickly cells, and low-cylindric basal cells. The basal cells were actively growing, assuming a spindle shape in places. Mitotic figures were seen occasionally among the palisades of basal cells. The characteristic epithelial products seen in the previous cases also were observed.

*Diagnosis.* Slowly growing, clear cell papillary carcinoma of hair-follicular origin.

#### CLINICAL AND PATHOLOGIC CONSIDERATIONS

The clinical features of the 4 cases are listed in Table I. All of the tumors grew slowly at the start. A history of rapid growth following improper surgical intervention was noted twice. The duration of the tumor varied from 2 to 20 years. All were located subcutaneously and were distributed in hair-bearing areas. The size of the tumors ranged from 1 to 12 cm. in diameter. A palpable lymph node was present in one instance but metastasis was not found.

All tumors were situated just beneath the epidermis. In 2 instances they protruded above the skin surface and exhibited an ulcerative,

nodular growth with purulent discharge. Cut section showed grayish neoplastic tissue forming papillary nodules or alveolar spaces. In areas there were irregular, yellowish, necrotic spots mingled with small cystic spaces filled with blood clot.

The histologic picture described above reveals several points of special interest.

*Cytologic Features.* Neoplastic cells were of three distinct types: clear, basal, and prickle. The cells constituting the major part of the tumor were polyhedral or cuboidal. They had distinct cell borders and small, eccentrically located, pyknotic nuclei. Some contained finely granular cytoplasm with clear vacuoles. Morphologically, these cells

TABLE I

*Summary of Clinical Data of 4 Cases of Clear Cell Papillary Carcinoma of Skin*

Case no.	Age	Sex	Duration of growth	Location	Size	Ulceration	Metastasis
1	20	M	Unknown	Subcutaneous tissue of left axilla	3 x 2 x 1 cm.	—	—
2	21	F	2 years	Subcutaneous tissue of left arm	8 x 1 x 1.5 cm.	—	—
3	48	F	20 years	Subcutaneous tissue of left thigh	12 x 10 x 10 cm.	+	—
4	48	M	2 years	Right upper eyelid	3 x 3.5 x 3.5 cm.	+	—

were very much like those which surround the internal root sheath cells above the neck of the hair papillae. In the neoplastic tissue there was also a palisade arrangement of basal cells closely associated with the clear cells. These cells correspond to the basal cells in palisade arrangement above the neck of the normal hair papillae. The prickle cells were found near the top of the tumor, which means that the superficial part of the tumor was better differentiated, a condition often seen also in the squamous cell carcinoma or nevus. Maximow and Bloom,<sup>5</sup> Mallory,<sup>6</sup> and Haythorn,<sup>7</sup> in their study of basal cell tumors of hair follicles, claimed that the basal cells are in reality true prickle cells.

*Papillary Arrangement.* All of the tumors showed papillary or alveolar growth and the papillae were closely arranged, leaving occasional crevices between them (Fig. 6). The fibrous stalk varied in amount and character and was made either of irregular bands of collagenous or hyalinized tissue or of acutely proliferating young fibrous tissue containing many capillaries. The significance of the papillary growth is self-evident, as the tumor is slowly growing and of a low degree of malignancy. The picture may become one of compact masses of neoplastic tissue, as shown in case 4.

*Epithelial Products.* In all instances, there were small cylindric formations which seemed to represent aborted or immature hair shafts. They could be distinguished from cornified pearls by their structure and from hyalinized substances by special staining methods (Fig. 10).

*Glycogen.* The glycogen content of the tumors was abundant, as shown by Best's carmine stain (Fig. 8). Lubarsch,<sup>8</sup> in his study of this subject, revealed that actively growing tumors of the skin contain more glycogen than slowly growing tumors, but that tumors arising from skin appendages such as hair follicles or sebaceous glands show the most glycogen in the cytoplasm of the tumor cells. Normal squamous epithelium contains small amounts of glycogen while epithelial cells of the mucous membranes, particularly the vaginal epithelium, show an abundant glycogen content.

*Epithelial Fibrils.* Under high magnification, intercellular bridges and fibrils were noted easily among the basal cells (Fig. 11). In comparing them with those of the normal hair matrix, no morphologic difference was found. The hair shaft is, in fact, composed of flattened or elongated epithelial cells with intercellular bridges running along the long axis of the matrix cells (Fig. 7).

## DISCUSSION

The origin of the so-called Krompecher's basal cell carcinoma<sup>2</sup> has long been a subject of controversy. Krompecher's original thesis was favored by Montgomery.<sup>9</sup> Mallory<sup>10</sup> and Haythorn<sup>7</sup> believed that all basal cell carcinomas of the skin are hair matrix tumors. Stout<sup>11</sup> and Boyd,<sup>12</sup> on the other hand, suggested that this tumor has multiple origins from the skin, hair follicles, and embryonic cell rests. In a recent study, Foot<sup>13</sup> expressed the opinion that the basal cell carcinomas of the skin originate in a distorted primordia of dermal adnexia. Embryonically, basal cells are mother cells of epidermis as well as of skin appendages. Therefore, these cells have multiple potentialities of development. In 1940, Traenkle<sup>14</sup> studied 63 examples of various epithelial neoplasms of the skin, in which 13 presenting pictures identical with Krompecher's basal cell carcinoma were found to show abortive attempts at hair follicle formation. This probably indicates that basal cell carcinomas have various morphologic differences and that the basis for such changes depends upon their types of differentiation.

The clinical features of clear cell papillary carcinoma are those of slowly growing tumors of low malignancy. The histologic pictures are unique. The papillary arrangement of clear cells, the palisade pattern of basal cells, the attempt at hair-shaft formation, and the close re-

semblance of the clear cells to the internal root sheath are evidence in favor of their origin from hair follicles. Compared with the usual hair follicle carcinoma or Mallory's hair matrix carcinoma, these tumors are histologically better differentiated and clinically more benign, although all belong to the same group.

That these tumors are not of sweat gland origin is self-evident. Their structure is quite different from that of sebaceous gland tumors. Hazen <sup>4</sup> and Eller <sup>16</sup> have stated that apocrine gland tumors show papillary cystic structure or diffuse anaplastic growth. Histologically, a well formed sebaceous tumor presents characteristic lobules <sup>16,17</sup> formed by squamous cells with central fatty change. Although in one of my cases there were foamy cells scattered here and there, one can hardly consider them as proper constituents of the tumor, particularly in the presence of infection and ulceration.

### SUMMARY AND CONCLUSION

Four cases of cutaneous carcinoma were characterized clinically by slow growth, subcutaneous location, and distribution on the hair-bearing areas of the body, and microscopically by papillary growth and palisade arrangements of low cylindric basal cells and rather disorderly arranged, glycogen-rich, clear cells. The close resemblance of the clear cells and the palisaded basal cells to the outermost layer of the internal root sheath cells, and the finding of immature hair shafts, suggest strongly that they originate in hair follicles.

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[ Illustrations follow ]



## DESCRIPTION OF PLATES

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### PLATE 13

- FIG. 1. Case 4. Cut surface of a clear cell carcinoma of the skin, showing the papillary and alveolar arrangement of the neoplastic tissue. Natural size.
- FIG. 2. Case 3. General topography of the tumor, showing its subcutaneous location, alveolar arrangement, invasion into the epidermis, and formation of many large and small cysts. Some of the rete pegs of the epidermis are greatly increased in length. Hematoxylin and eosin stain.  $\times 7$ .
- FIG. 3. Case 1. The characteristic palisade arrangement of low-cylindric cells around the vascular stroma is shown. Hematoxylin and eosin stain.  $\times 155$ .
- FIG. 4. Case 2. The clear cells of the tumor present an alveolar arrangement. Hematoxylin and eosin stain.  $\times 155$ .
- FIG. 5. Case 3. The palisade arrangement of the tumor cells may be seen. Hematoxylin and eosin stain.  $\times 155$ .

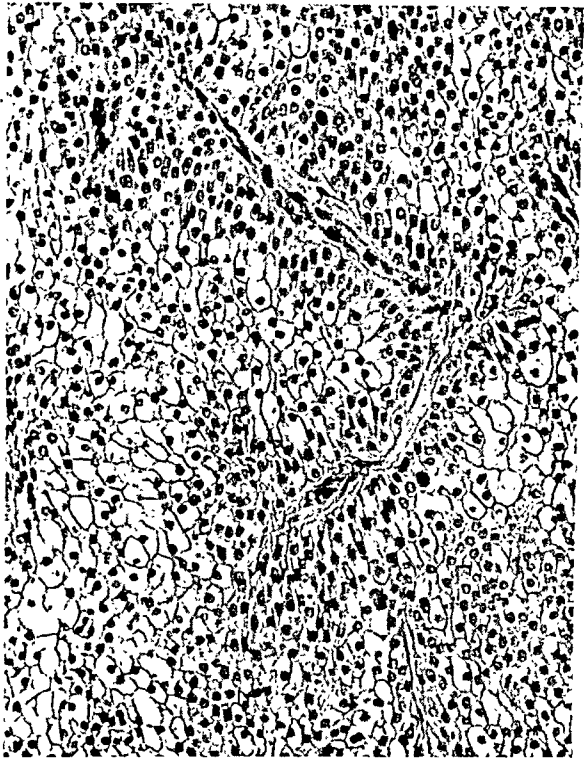
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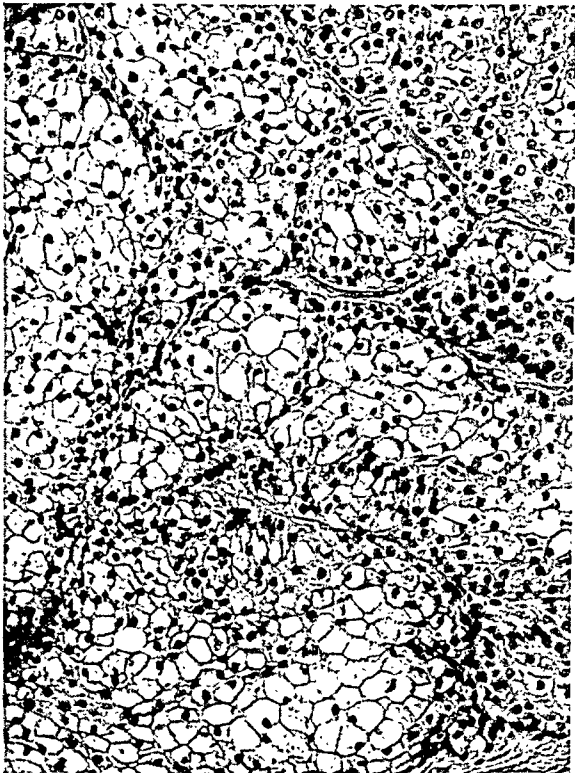
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#### PLATE 14

FIG. 6. Case 1. A pseudocyst lined by two layers of flattened epithelial cells. Papillary growth of tumor in lumen. Hematoxylin and eosin stain.  $\times 155$ .

FIG. 7. Epithelial fibrils in normal hair matrix cells. Foot's silver method.  $\times 1250$ .

FIG. 8. Case 3. Glycogen in tumor cells. Best's carmine stain.  $\times 155$ .

FIG. 9. Immature hair shaft in normal skin. Hematoxylin and eosin stain.  $\times 570$ .

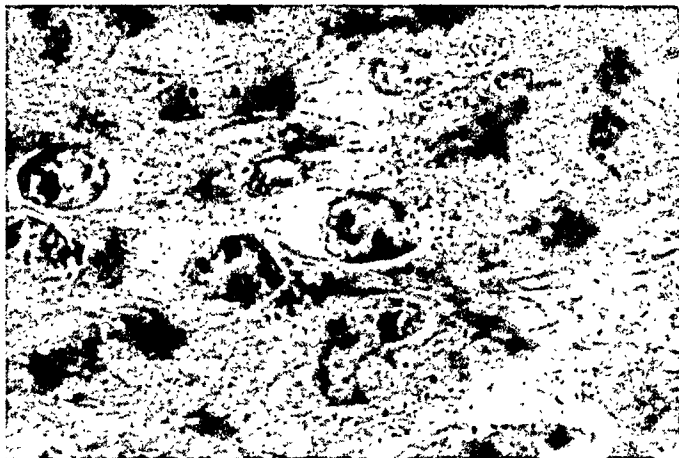
FIG. 10. Case 1. Immature hair shaft in tumor. Hematoxylin and eosin stain.  $\times 570$ .

FIG. 11. Epithelial fibrils in tumor cells. Foot's silver stain.  $\times 1290$ .

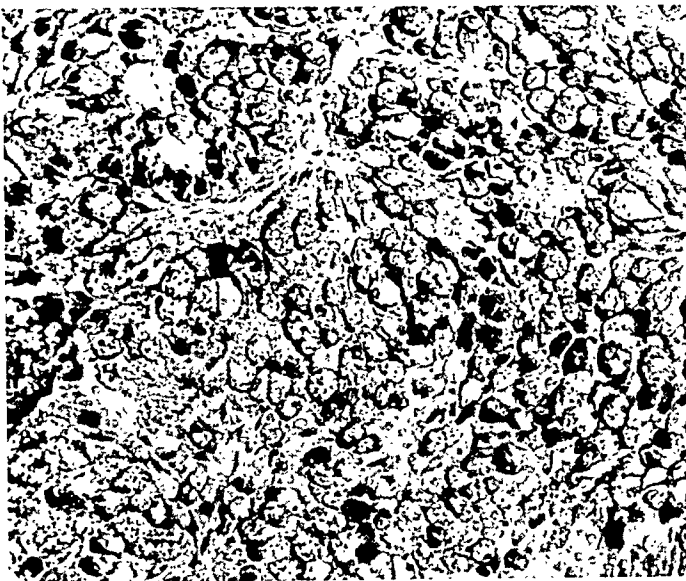
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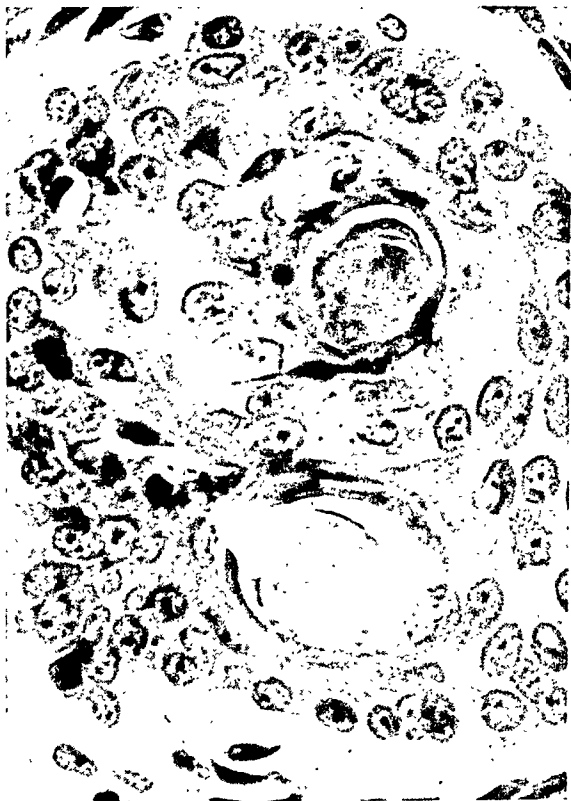
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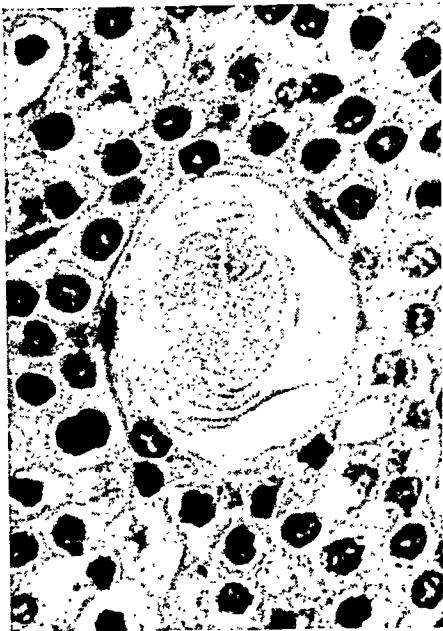
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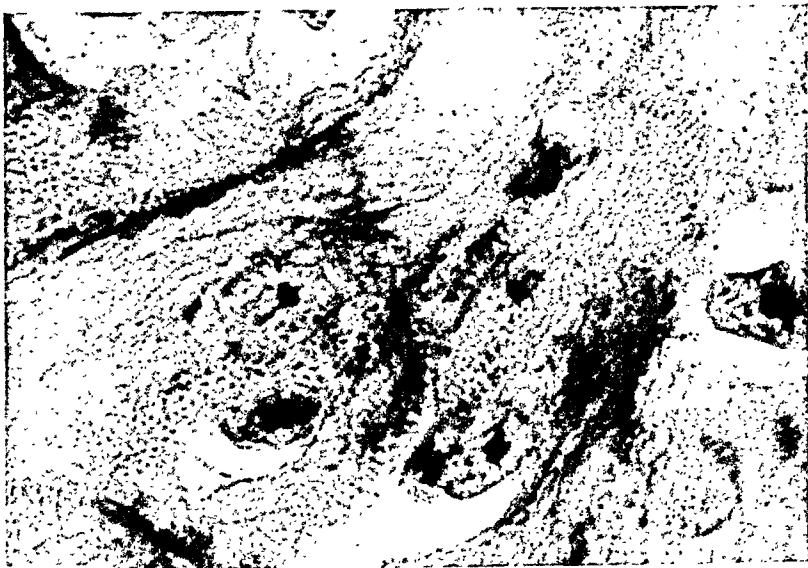
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# PRIMARY AMYLOIDOSIS, WITH REPORT OF SIX CASES \*

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Primary systemic amyloidosis is an unusual disease, there being only slightly more than 50 cases reported in the literature. It differs from the more common secondary amyloidosis in several respects: (1) Absence of the generally considered specific etiologic factors such as tuberculosis or chronic suppuration, (2) often minimal involvement of the liver, spleen, kidneys, and adrenal glands, which are ordinarily the sites of maximal deposition in secondary amyloidosis, (3) usually considerable deposition in the heart, lungs, skin, striated muscles, and other tissues not often involved in the secondary type, (4) often atypical reactions to the specific amyloid stains, and (5) the occasional occurrence of amyloid tumors.

Wild,<sup>1</sup> in 1886, is credited with reporting the first case of primary amyloidosis. Lubarsch<sup>2</sup> reported 3 cases in 1929, and suggested criteria for the diagnosis. In 1939 Koletsky and Stecher<sup>3</sup> reviewed the literature, tabulated data on 23 cases, and reported a case of their own. Lindsay and Knorp,<sup>4</sup> in 1945, similarly tabulated data on 16 additional examples and reported a case. In 1946 Lindsay<sup>5</sup> reported another case of primary amyloidosis and included those of Brown and Selzer,<sup>6</sup> Golden,<sup>7</sup> Bannick, Berkman, and Beaver<sup>8</sup> and a second case of Pick's<sup>9</sup> in an analysis of the total of 45 cases. He stressed the importance of the cardiac lesions in this disease and pointed out that 23 patients showed signs of cardiac failure during their illness and that, in 18 of these, cardiac failure was considered the immediate cause of death.

In reviewing the literature, it was found that additional examples of this disease have been reported by Barnard, Smith, and Woodhouse<sup>10</sup> (2 cases), Kernohan and Woltman,<sup>11</sup> Bürümcekci,<sup>12</sup> Fowler,<sup>13</sup> Soisalo and Ritama,<sup>14</sup> Ferris,<sup>15</sup> Bell,<sup>16</sup> Eisen<sup>17</sup> (2 cases) and Ranstrom.<sup>18</sup> This makes a total of 57 cases of primary systemic amyloidosis found in the literature. There are, perhaps, others that have been overlooked. It is noteworthy that differences in terminology make for difficulty in locating case reports. Atypical amyloidosis,<sup>9</sup> paramyloidosis,<sup>12</sup> unusual amyloid deposits,<sup>2</sup> idiopathic amyloid disease,<sup>13</sup> and amyloid neuritis<sup>11</sup> are some of the titles under which reports of cases of primary systemic amyloidosis have been found.

Of all the criteria given for the diagnosis of primary systemic amy-

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lloidosis, absence of pre-existing or etiologic disease is the only constant clinical and pathologic finding. The staining reactions of the primary amyloid as well as its distribution overlap those seen in secondary amyloidosis. Because of these facts, opinions differ regarding what constitutes an example of primary systemic amyloidosis. Some writers have believed that the cases of Bell <sup>16</sup> and Fowler <sup>17</sup> should not be included.

Of the 57 cases of primary systemic amyloidosis in the literature, information regarding sex and age was available in 55. The average age was 55 years, the oldest patient being 80 and the youngest, 15 years. No significant sex predilection was noted, 30 of the patients were males and 25, females.

More or less complete necropsy reports were available in 54 cases. Cardiac amyloid infiltration was specifically mentioned in 46 instances, and in 25 of these, myocardial insufficiency was considered the cause of death. Involvement of the gastro-intestinal tract was mentioned in 33 cases. In 2 cases <sup>2,10</sup> a clinical diagnosis of carcinoma of the stomach was made. In a third,<sup>7</sup> gastric resection was performed, and it was discovered that diffuse amyloid infiltration had caused pyloric obstruction and hematemesis. Many of the patients with amyloid in the gastro-intestinal tract presented no definite symptoms referable to this phase of the disease, but 5 of them had serious hemorrhage from the gastro-intestinal tract.<sup>2,7,10-21</sup> Amyloid was found in the tongue of 26 patients, in most of whom macroglossia was noted clinically. Pulmonary involvement was noted in 24 cases, in one of which <sup>12</sup> severe amyloid infiltration of the lungs resulted in an extensive shadow in the roentgenogram and was associated with right ventricular hypertrophy. Infiltration of amyloid in striated muscle, which was observed in at least 19 instances, has been blamed for weakness, fatigue, and limitation of motion.<sup>4</sup> Amyloid deposits were present in the skin of about one-fourth of the cases, an important diagnostic feature. It is worthy of note that involvement of the liver and kidneys was found in 21 cases and splenic involvement in 20. In one instance,<sup>8</sup> massive amyloidosis of the liver simulated hepatic cirrhosis clinically. Involvement of bones and joints has been reported.<sup>4,22</sup> Involvement of small arteries and veins of many organs was seen commonly. In one of these, obstruction of vasa nervorum by amyloid in their walls resulted in clinical signs due to ischemic neuropathy.<sup>11</sup> In the case reported by Götze and Krücke,<sup>23</sup> amyloid was present in the peripheral nerves and in the vessels of the brain. This case was probably an example of primary amyloidosis in spite of the associated suppurative renal disease. Neurologic signs due to amyloid occurred in the case described by De Navasquez and Treble.<sup>24</sup> In this brief re-

view, many of the less commonly involved sites have not been mentioned. The intravenous Congo red test may give positive<sup>22</sup> or negative<sup>7</sup> results in cases in which extensive involvement occurs.

Of the 44 cases of amyloid disease in which necropsy was performed at the Mayo Clinic during the 25-year period 1922 through 1946, 7 were examples of primary systemic amyloidosis.\* In an eighth case, necropsy was done in 1947. Six of these patients were men and 2 were women. Their ages varied from 40 to 75 years, the average being 56 years. The interval between the onset of symptoms and death varied from 8 months to 4 years. Two of these cases have been reported previously.<sup>8,11</sup> A review of the 6 cases not previously reported follows:

### REPORT OF CASES

#### *Case 1*

A white woman, 75 years old, who registered at the Clinic on April 21, 1938, gave a history of ankle edema of 2 years' duration. She had lost 30 lb. (13.6 kg.) in the 10 months preceding registration and had vomited occasionally during the last 2 months. The blood pressure was 140/80 mm. of Hg. The liver was enlarged and firm. Albuminuria was graded 2 (on the basis of 1 to 4, in which 1 represents the lowest concentration of albumin). The hemoglobin was 12 gm. per 100 cc.; erythrocytes, 4,860,000; leukocytes, 10,200 per cmm. A flocculation test for syphilis gave negative results. A roentgenogram of the chest showed enlargement of the heart and congestion in the bases of both lungs.

During the next 2 months dyspnea on exertion, orthopnea, and nocturnal dyspnea developed. Large, discrete cervical nodes were noted in June, 1938, when there was moderate pitting edema of the lower extremities. An electrocardiogram showed a rate of 98 per minute, slurred QRS<sub>1</sub> and QRS<sub>3</sub>, notched QRS<sub>2</sub>, isoelectric T<sub>2</sub>, inverted T<sub>3</sub>, and low amplitude QRS in all leads. The patient failed rapidly and died on June 29, 1938.

At necropsy the peritoneal cavity contained about 300 cc. of clear fluid and each pleural cavity about 2 l. of similar fluid. The heart weighed 468 gm., and the right auricle and right ventricle were moderately dilated. The spleen weighed 144 gm.; the liver, 1850 gm.; and the kidneys, 255 gm. The cervical, mediastinal, and abdominal lymph nodes were firm, discrete, and measured up to 4.0 cm. in diameter. Their cut surfaces were glistening and somewhat translucent.

On microscopic study the walls of the small arteries and arterioles of all tissues examined were found to be infiltrated by a homogeneous, amorphous material which stained pink with hematoxylin and eosin. This amyloid was deposited in the media of the vessels and resulted in marked decrease in the caliber of the lumina of many of the affected vessels. The small vessels of the heart and lungs were most severely

\* These data are based on a study of material in the Section on Pathologic Anatomy of the Mayo Clinic. The study was made under the guidance of Dr. J. W. Kernohan.



involved, but the vascular amyloid was prominent in all tissues examined. In the myocardium there were areas where a moderate amount of the substance had been deposited on the reticulum between muscle bundles and nutrient capillaries. Variable degrees of myocardial atrophy had occurred in these areas. Parenchymal involvement was prominent in the liver; the hepatic cords were markedly replaced by amyloid which had been laid down on the reticulum between the sinusoids and the cords of hepatic cells. The spleen was involved diffusely and severely. The lymph nodes were affected to such a degree that the normal architecture was barely recognizable. Lipoid tissue in association with lymph nodes and elsewhere showed a thin deposit of amyloid on the surface of individual fat cells. The gallbladder was moderately thickened by masses of the material in the subepithelial tissue. The gastro-intestinal tract, genito-urinary tract, and pancreas showed involvement of only the small blood vessels. There was a small amount of amyloid between the thyroid acini. Mild involvement of the vessels in the striated muscle was noted. In some areas amyloid was surrounded by giant cells of foreign body type and by lymphocytes. Sections of marrow from the vertebrae and ribs showed no abnormality.

### *Case 2*

A white man, 73 years of age, registered at the Clinic on January 19, 1940, with a 6 months' history of dyspnea on exertion, edema of the ankles, palpitation, and anorexia. Two months previously, when he had been hospitalized for congestive cardiac failure, an electrocardiogram showed a rate of 81 per minute, incomplete left bundle-branch block (QRS, 0.12 second), inverted  $T_1$ , delayed atrioventricular conduction (P-R, 0.24 second) and diphasic  $P_3$ . A small, high-grade epithelioma of the base of the tongue had been successfully treated with radium in 1925. In May, 1939, his blood pressure had been 140/66 mm. of Hg.

When admitted to the Clinic on January 19, 1940, the blood pressure was 109/65 mm. of Hg. Râles were heard over the bases of the lungs and the liver was palpated 4 cm. below the right costal margin. A flocculation test for syphilis gave negative results. Albuminuria was graded 2, and the blood urea level was 52 mg. per 100 cc. After rest in bed and the administration of diuretics, a transurethral prostatic resection for urinary obstruction was done on January 22. After prostatic resection, the systolic blood pressure remained under 100 except on one occasion when it rose to 110. There was continuous oliguria and the value for blood urea rose to 272 mg. per 100 cc. on February 16. On February 9, the day after the patient was given a blood transfusion of 250 cc., he became icteric. The level of direct serum bilirubin rose to 10 mg. per 100 cc. Marked dependent edema developed and the patient died on February 17, 1940.

At necropsy, dependent edema and icterus were noted. The peritoneal cavity contained about 1500 cc. of clear, yellow fluid. The visceral and parietal pericardial surfaces were lined by yellow, fibrinous material. The heart weighed 590 gm., and all of its chambers were

moderately dilated. There was moderate coronary atherosclerosis. The spleen weighed 485 gm.; the liver, 2300 gm. They were firmer than is normal. The kidneys weighed 342 gm.

All of the organs examined, except the bone marrow and spinal cord, showed vascular amyloid infiltration similar to that seen in case 1. The media of the small arteries and arterioles was the site of deposition. The most significant involvement was in the myocardium where many of the small vessels were affected. There was also severe, diffuse deposition of amyloid on the reticulum of the myocardium and atrophy of associated muscle fibers. In the gastro-intestinal tract there was amyloid in the walls of the small vessels. Some of these vessels showed resultant narrowing of their lumina. The intestinal mucosa was slightly infiltrated. In the liver there was considerable involvement of small arteries, some of which showed moderate narrowing. There was atrophy of hepatic cells in the peripheral portions of the hepatic lobules with condensation of the reticulum in these areas. In this condensed reticulum there was a small amount of poorly staining amyloid. There was slight to moderate deposition of amyloid in the walls of the small vessels of the kidneys, pancreas, spleen, adrenal glands, lymph nodes, thyroid, prostate, and seminal vesicles. In the stroma of the prostate and thyroid there were moderate, diffuse amyloid deposits. There was a thin layer of the material beneath the epithelium of the tongue. Sections of the bone marrow appeared normal. It is noteworthy that tissue taken for biopsy of the original tumor of the tongue showed no amyloid. A very small area of adenocarcinomatous tissue without suppuration was noted in the microscopic sections of the posterior portion of the prostate. It is extremely unlikely that the cured carcinoma of the tongue or the early carcinoma of the prostate could be considered causes of amyloidosis, especially since distribution of the amyloid was of the primary type.

### *Case 3*

A white man, 56 years old, registered at the Clinic on May 4, 1943, because of a loss of 50 lb. (22.7 kg.) in the preceding year, anorexia for 3 months, jaundice for 2 months, and generalized pruritis and clay-colored stools for 10 days. In February, 1943, he had undergone transurethral prostatectomy. For 18 months he had noted dyspnea on exertion. He was moderately icteric and had "spider" angiomas on the head and neck. Axillary, cervical, and inguinal nodes were enlarged. The liver and spleen were enlarged. There was dependent edema. Râles were heard over the bases of the lungs. The blood pressure was 130/70 mm. of Hg. A flocculation test for syphilis gave negative results. The specific gravity of the urine was 1.010. Albuminuria was graded 4. The value for blood urea was 76 mg. per 100 cc.; for creatinine, 3.6 mg.; for direct bilirubin, 5.4 mg.; for indirect bilirubin, 3.0 mg.; for plasma proteins, 7.8 gm.; the albumin-globulin ratio was 1:1.47, and the prothrombin time (Quick) was 25 seconds (normal, 18 seconds). An electrocardiogram showed

a rate of 82, sinus rhythm, notched QRS<sub>3</sub>, left axis deviation, notched P<sub>3</sub>, inverted T<sub>1</sub>, and diphasic T<sub>2</sub>. Scattered cutaneous ecchymoses, shifting dullness in the abdomen, and stupor developed. The blood urea rose to 92 mg.. Death occurred on May 10, 1943.

At necropsy, moderate jaundice and edema were found. The peritoneal cavity contained about 1 l. of serosanguineous fluid. The right pleural cavity contained about 1 l., and the left about 500 cc., of clear, yellow fluid. The heart weighed 560 gm. The spleen weighed 975 gm. and was soft. The liver weighed 4000 gm., and its consistency was normal. The kidneys weighed 538 gm. There was slight enlargement of the lymph nodes throughout the body.

Microscopic examination revealed diffuse, severe infiltration of the spleen with amyloid. The hepatic parenchyma was diffusely infiltrated, and the adrenal cortex and renal glomeruli were severely involved. There was moderate infiltration around the renal tubules. Throughout the ventricles and atria there was slight to moderate infiltration with amyloid which was deposited on the reticulum surrounding the muscle fibers. In some areas there was mild atrophy of associated myocardial fibers. A small amount of the material was present beneath the endocardium of the auricles and in the tricuspid valve. With the exception of the skin and aorta, there was mild to moderate involvement of the small vessels of all tissues examined. A slight, diffuse deposition of amyloid was found in the smooth muscle of the gastro-intestinal tract. The lymph nodes were moderately and diffusely infiltrated. There was a small amount of amyloid in the alveolar walls of the lung and on the reticulum of striated muscle. The interstitial tissue of the thyroid, prostate, and testes contained considerable amyloid. There was slight thickening of the walls of a few capillaries in the bone marrow due to the deposition of amyloid. In several locations the amyloid appeared in adipose connective tissue where it was on the surface of individual fat cells. The bone marrow showed no evidence of myeloma.

#### *Case 4*

A white man, 67 years of age, registered at the Clinic on December 1, 1943, with the complaint of pain in the left side of the chest on exertion during the previous 4 years. For 9 months he had had severe dyspnea, dependent edema, and weakness which confined him to bed most of the time. Three weeks before admission, abdominal swelling, nausea and vomiting had developed. When examined he was dyspneic. Râles were heard over the bases of the lungs. Heart tones were distant. The blood pressure was 102/72 mm. of Hg. There was questionable ascites, and moderate pitting edema of the feet, ankles, and abdomen. Albuminuria was graded 2. The hemoglobin was 15.8 gm.; leukocytes, 7,600. The value for blood urea was 76 mg. and the creatinine, 2.7 mg. A flocculation test for syphilis gave negative results. A roentgenogram of the chest showed fluid in the left base. An electrocardio-

gram disclosed an auricular rate of 136 and a ventricular rate of 80, slurred QRS<sub>1</sub> and QRS<sub>2</sub>, notched QRS<sub>3</sub>, low amplitude QRS in leads I, II, and III, right axis deviation and indefinite T waves. The sedimentation rate was 2 mm. in 1 hour. The value for blood urea rose to 122 mg. and for creatinine to 3.9 mg. Anasarca became severe, and death occurred on December 15, 1943.

At necropsy about 2500 cc. of cloudy, yellow fluid were found in the peritoneal cavity. The right pleural cavity contained about 1000 cc., and the left pleural cavity about 1500 cc., of similar fluid. There were about 300 cc. of clear, yellow fluid in the pericardial sac. The heart weighed 662 gm. The spleen weighed 667 gm. and was firmer than is normal. The liver weighed 1865 gm. and showed chronic passive congestion. The kidneys weighed 304 gm.

Microscopic examination revealed infiltration of most of the organs with amyloid. The heart showed moderate to severe involvement of the ventricular myocardium and slight involvement of the auricles. The walls of the small vessels of the heart and the subendocardial tissue of the atria contained small amounts of the substance. The small vessels of the lungs contained moderate, and the alveolar walls slight, amounts of amyloid. The adrenals and spleen showed marked infiltration and the renal glomeruli moderate infiltration. The walls of the small blood vessels of every organ of the neck, chest, and abdomen showed slight to moderate amyloid infiltration. The hepatic parenchyma was uninvolved. Fat cells in several locations were surrounded by "amyloid rings." A thin collar of amyloid surrounded the acini of Brunner's glands. The thyroid acini were separated by moderate amounts of the substance. Vessel walls in the testes contained amyloid. The diaphragm showed involvement similar to that of the heart. In several sites the amyloid was surrounded by a foreign body reaction consisting of giant cells and a few lymphocytes. A section of vertebral marrow appeared normal.

### *Case 5*

A white woman, 44 years old, who registered at the Clinic on October 9, 1944, had had edema of the lower extremities and abdomen for 7 months. In the past 3 months she had noted abdominal swelling and had had one paracentesis abdominis. Recently, marked weakness and slight dyspnea had developed. The blood pressure was 110/80 mm. of Hg. Breath sounds were diminished over the lower lung fields. The abdomen was markedly distended and the lower parts of the trunk and legs were edematous. Albuminuria was graded 2 to 4. The hemoglobin was 12.1 gm.; erythrocytes, 4,130,000; and leukocytes, 9,400. The electrocardiogram showed a rate of 72 per minute, low amplitude QRS in leads I, II, and III, low amplitude T<sub>2</sub> and inverted T<sub>3</sub>. The blood urea level was 24 mg. The serum protein level was 4.2 gm., with an albumin-globulin ratio of 1.0:1.4. One hour after injection of 5 mg. of bromsulfalein per kg. of body weight, more than 40 per cent of the substance remained in the blood stream. On October 12, 5000 cc. of cloudy peritoneal fluid were removed. Persistent oliguria began and did not respond

to saline and mercurial diuretics. On October 23, the value for blood urea had risen to 192 mg. and for creatinine, to 0.3 mg. She had extreme drowsiness beginning on October 19, and on this date the blood sugar level was 35 mg. per 100 cc. Only slight improvement followed intravenous administration of glucose. Coma supervened, and death occurred on October 24, 1944.

At necropsy there was brownish yellow discoloration of the skin. Evidence of residual ascites was present. The heart weighed 220 gm. The spleen weighed 480 gm. and was firm. The liver weighed 2800 gm. and was firmer than is normal. There were scattered submucosal petechiae throughout the gastro-intestinal tract. The kidneys weighed 427 gm.

Microscopic examination revealed marked amyloid infiltration of the liver, spleen, kidneys, and adrenal glands. The hepatic parenchyma was almost completely replaced with amyloid. Renal glomerular infiltration left little space for blood flow through the capillaries. The splenic involvement was diffuse or "bacony" in type. Throughout the myocardium there was moderate, diffuse amyloid infiltration, with deposition on the reticulum. There was patchy atrophy of associated muscle fibers. A thin layer of amyloid lay beneath the endocardium of the auricles. The small vessels of the heart, as well as of every organ of the chest and abdomen, showed slight to moderate thickening of the media as a result of the presence of amyloid. There was slight infiltration of the pulmonary alveolar walls. Several lymph nodes and the gastric mucosa showed slight diffuse involvement. The fat in the region of the pancreas and in the epicardium showed a thin layer of amyloid surrounding individual cells. The bone marrow was normal grossly.

### *Case 6*

A white man, 40 years of age, registered at the Clinic on November 9, 1947, because of edema of the ankles and ascites of 6 weeks' duration. In July, 1947, he had had paresthesia with slight swelling of the hands and forearms. Two months later he began having dyspnea on exertion and noted pain in the back. On examination, the blood pressure was 102/80 mm. of Hg.; pulse, 120. The tongue was red and the submaxillary glands were large. Moist râles were heard over the bases of the lungs. The liver was enlarged and the abdomen distended. There was marked edema of the lower extremities. The patient died suddenly before laboratory study was made.

At necropsy there was marked edema. The peritoneal cavity contained about 4000 cc. of clear, amber fluid. The right pleural cavity contained about 300 cc., and the left about 200 cc., of similar fluid. The heart weighed 525 gm. and was very firm. Two small mural thrombi were found in the right auricular appendage. The spleen weighed 535 gm. and was very firm. Its cut surface was homogeneous, grayish purple, and showed no markings. The liver weighed 2485 gm., and the

centers of the lobules were dark. The wall of the stomach was somewhat thicker than is normal. The tongue was moderately enlarged as were the submaxillary and sublingual salivary glands. The right kidney weighed 165 gm. and its pelvis contained a small amount of inspissated, sandy material. The left kidney weighed 120 gm. Its parenchyma was thin over dilated calyces which, along with the renal pelvis and the dilated left ureter, contained inspissated, sandy material. The heart and spleen showed the characteristic mahogany brown color on application of iodine.

Microscopic study of the heart revealed marked infiltration of the myocardium of all four chambers by a homogeneous material which stained pink with eosin. There was marked associated atrophy of muscle fibers (Fig. 1). The spleen was diffusely infiltrated with amyloid to the extent that normal architecture was barely recognizable. The tongue showed infiltration between its mucous glands and muscle fibers (Fig. 2). The connective tissue between the lobules of the submaxillary and sublingual glands was markedly thickened as a result of amyloid deposits, and smaller amounts of interacinar amyloid were seen. The pancreas presented a similar picture. In the esophagus and stomach there was mild infiltration of the muscularis. The gastric mucosa contained a small amount of the substance. There was slight patchy infiltration in the lungs, chiefly in the vicinity of the bronchi. The thyroid was slightly involved. The wall of the urinary bladder and the prostate contained small amounts of amyloid between the muscle bundles. Lymph nodes from several sites showed slight amyloid infiltration and several lymph nodes showed none. Adipose connective tissue from several locations contained amyloid which surrounded individual fat cells. In the left kidney there was evidence of hydronephrotic atrophy and healed pyelonephritis. There was no current, active, inflammatory process in the kidneys that could be considered as the cause of amyloidosis. In addition, the amyloid distribution was not of the secondary type. The amyloid in this case showed only very slight metachromasia with the methyl violet stain but stained well with Congo red in some locations. No evidence of myeloma was found in sections of the vertebral marrow, but one section contained a lymph follicle which was slightly infiltrated with amyloid.

#### COMMENT

In these 6 examples of systemic amyloidosis there were no demonstrable preceding or etiologic diseases. The staining reactions of the material were similar in all instances, although they were much more

variable than in secondary amyloidosis. The amyloid was homogeneous and amorphous and stained pink with hematoxylin and eosin. In all instances there was definite metachromasia in at least some of the sections stained with methyl violet, although the intensity of the reddish violet color varied markedly in different cases. With the Congo red stain the amyloid assumed a red color, but this stain was not as satisfactory as methyl violet in differentiating amyloid from dense connective tissue. For this differentiation the van Gieson stain was extremely valuable. With it the amyloid stained yellow or very faintly pink, whereas hyaline or collagenous connective tissue of similar density stained brilliant red.

It is apparent that the distribution of amyloid in these cases differs from that usually described for secondary amyloidosis, especially in the constancy of involvement of the heart and the frequency of involvement of striated muscle, lungs, and small blood vessels throughout the body. The cases in which involvement of the spleen, adrenals, liver, and kidneys occurred demonstrate, however, that amyloid distribution of the so-called secondary type is overlapped in primary systemic amyloidosis.

The heart contained significant amounts of the material in all 6 examples of primary systemic amyloidosis. Grossly, the nature of the disease was not always evident. In 2 cases, small, translucent, sub-endocardial masses of amyloid were seen. The infiltration in the hearts was diffuse, involving the myocardium of the auricles and ventricles. It consisted chiefly of two main types. Patchy or diffuse deposition of amyloid on the reticulum surrounding the muscle bundles was associated with partial atrophy of the encased muscle fibers (Fig. 1). Amyloid was deposited also in the walls of the small arteries, arterioles, and veins of the myocardium, sometimes markedly decreasing their lumina. A small amount of amyloid was found beneath the atrial endocardium in all 6 hearts. Small masses of amyloid were found within the tricuspid valve in case 3. In only 2 cases was there any significant coronary atherosclerosis and this was moderate. The vessels and fat of the epicardium contained variable but small amounts of amyloid.

The hearts were larger than is normal except in case 5. The average cardiac weight was 505 gm. Grossly, they showed concentric hypertrophy. This hypertrophy occurred in patients whose blood pressure was normal or subnormal. The highest pressure (140/80 mm. of Hg.) was observed in case 1. It appears that amyloidosis produces large hearts in the absence of valvular defects or hypertension. There were low amplitude QRS complexes in the electrocardiograms of 3 cases.

Cardiac decompensation supplied the main clinical signs and symptoms as well as the cause of death in 4 patients.

The involvement of small arteries, arterioles, and small veins of many organs was a prominent feature in several of these cases. The material was deposited in the media. In mild degrees of the process it could be seen as a thin layer surrounding the smooth muscle cells. As the disease progressed, the muscle cells became separated by the material and some decrease in size of the vascular lumen resulted (Fig. 3). With severe involvement of the vessel, the lumen was markedly narrowed by the thickened media and amyloid often extended into the adventitia.

The liver showed marked parenchymal amyloid infiltration in 2 cases. This resulted in severe atrophy of the hepatic cords (Fig. 4). The material was deposited on both sides of the reticulum fibers lying between the sinusoids and the cords of liver cells. The exact site of deposition in the liver and in the heart was demonstrated by staining sections for reticulum and then with methyl violet. In one case only slight, and in another moderate, amounts of amyloid were present in the hepatic parenchyma. In only 2 cases was the hepatic parenchyma free of amyloid. The small vessels, especially the arteries, of the portal triads showed slight to severe infiltration of their walls in all cases. The livers in the cases of moderate or severe amyloidosis were firm and rubbery and had an average weight of 2880 gm.

In case 5, with severe hepatic amyloidosis, a clinical diagnosis of cirrhosis of the liver was entertained. A bromsulfalein test of liver function revealed retention of more than 40 per cent of the dye at the end of 1 hour. In case 3, in which there was moderate involvement of the liver, obstructive jaundice was the clinical diagnosis and the direct serum bilirubin rose to 5.4 mg. and the indirect to 3.0 mg. These two patients had jaundice, ascites, and edema of the legs. In case 3, the firm, enlarged liver was noted clinically. The clinical picture was complicated by severe renal amyloidosis with marked proteinuria in these 2 cases. In both there was a reversed albumin-globulin ratio with blood protein levels of 7.8 gm. and 4.24 gm., respectively, and there was considerable terminal azotemia.

Involvement of the small blood vessels of the kidneys was noted in 5 cases. In 2 patients whose signs and symptoms suggested primary hepatic disease, the glomerular amyloidosis was severe, that is, the diameter of the glomerular capillary lumina was greatly decreased. These patients had marked albuminuria and the changes in blood protein mentioned above. In these 2 patients the terminal levels of blood urea were 92 and 192 mg., respectively. The coexisting hepatic and



cardiac disease made it difficult to evaluate the clinical significance of the renal lesion. In the remaining 4 patients, deposit of glomerular amyloid was moderate in one, mild in one, and absent in 2.

The spleen was severely infiltrated with amyloid in 5 instances. Diffuse "bacony" involvement of the splenic cords with loss of splenic corpuscles had occurred (Fig. 5). These spleens were firm and their average weight was 560 gm. Again, portal obstruction and cardiac insufficiency may have affected their size. Slight splenic amyloid infiltration was noted in the remaining case. Rather slight involvement of the walls of the small blood vessels was seen in all of the spleens.

The gastro-intestinal tract was slightly to moderately involved in all 6 cases. The small blood vessels were constantly affected. In all cases, amyloid was deposited diffusely, in slight to moderate degree, on the smooth muscle cells of the muscularis mucosae and muscularis propria. Mild involvement of the mucous membrane was seen in only 3 cases. No gross alterations of the gastro-intestinal tract due to amyloid were observed. Gastro-intestinal symptoms, such as constipation, diarrhea, anorexia, and vomiting, occurred in 4 cases, but, again, the problem of evaluation in the face of coexisting cardiac, hepatic, and renal disease is obvious.

Amyloid deposition of moderate to severe degree was observed in or around the small vessels of the lungs in 5 cases (Fig. 6). Slight patchy deposits around the capillaries of the alveolar walls were observed in 4 cases. In one case no amyloid was found in the lungs. In none was the pulmonary amyloidosis of apparent clinical importance.

The small vessels around and in the lymph nodes were slightly to severely affected in all but one case (Fig. 7). Diffuse infiltration of the nodes similar to that seen in the "bacony" spleens occurred in 5 cases and, in 2 of these, it was responsible for clinically evident enlargement of the cervical nodes. The diagnostic value of biopsy of lymph nodes should be borne in mind.

There was slight to moderate infiltration of the walls of the small blood vessels in striated muscle in the 5 cases in which samples were available for study. In 3 of these there was a small amount of amyloid laid down on the reticulum surrounding the muscle fibers. No definite symptoms can be ascribed to the infiltration in the muscle, but it does serve to emphasize the diffuse deposition in primary systemic amyloidosis.

The adrenal cortex was largely replaced with amyloid in 3 cases. The deposition was similar to that seen in secondary amyloidosis. The periadrenal and medullary small vessels in 5 cases were affected. Peri-

adrenal fat was infiltrated in the sixth case. Signs pointing to adrenal insufficiency were not observed.

The blood vessels of the thyroid were involved in 4 of the 5 cases in which the gland was studied. Diffuse infiltration between the acini occurred in all 5 (Fig. 8). In one of these the acini were widely separated, and in the others moderately separated, by the substance.

In none of these 6 cases was macroglossia noted clinically, although the tongue was found to be enlarged at necropsy in one. In only 2 instances was the tongue examined at necropsy. These showed microscopic evidence of moderate amounts of amyloid in the small vessels, around some of the mucous glands, and on the reticulum surrounding some of the muscle fibers.

The walls of the blood vessels in the pancreas were involved in all but one instance. In 4 of the 6 cases, amyloid was found in the pancreatic interstitial tissue. In the 4 men the small prostatic vessels contained amyloid in their walls. In 3 instances, moderate diffuse infiltration in the smooth muscle of the gland was seen. In all 6 cases, amyloid was seen in the adipose tissue of such sites as the epicardium, pancreas, and periadrenal regions. The amyloid appeared to be laid down upon the surface of the fat cells, giving their walls a thickened appearance. These rings of amyloid on fat cells have been considered characteristic of primary amyloidosis.

No skin lesions were observed clinically except purpura, which occurred in one patient. Sections of skin from the trunk were studied in 3 cases and no abnormality was noted. The cause of the purpura was not determined. Amyloid infiltration of small blood vessels of the skin has been held responsible for the purpura by some observers.<sup>2</sup>

#### SUMMARY AND CONCLUSIONS

The 6 cases of primary systemic amyloidosis reported in this paper demonstrate the remarkably diffuse involvement of mesodermal structures which occurs in this disease. The organs most severely affected in secondary amyloidosis are, however, not immune to infiltration in the primary type.

The clinically important lesions were cardiac in 3 cases, which is in conformity with the findings in the majority of reported cases of primary systemic amyloidosis.

An unusual syndrome was presented in the 2 cases in which primary hepatic disease was simulated. The association of signs of hepatic disease and of renal disease in these is noteworthy.

That macroglossia as a diagnostic mainstay in primary systemic

amyloidosis has been overrated is evidenced by its absence in 5 of these cases. It was observed in less than one-third of the cases reported in the literature.

The identity of the homogeneous, amorphous, pink-staining material seen in sections stained with hematoxylin and eosin should be confirmed by the use of special stains. In these 6 cases the amyloid showed definite metachromasia with methyl violet. The affinity of amyloid for Congo red in primary amyloidosis was not as great as that observed in secondary amyloidosis. The van Gieson connective tissue stain is valuable in differentiating amyloid from hyaline or collagenous connective tissue of similar density.

Biopsy of affected organs is the only certain diagnostic procedure in the cases in which the amyloid lacks affinity for intravenously injected Congo red.

No common denominator in these cases furnished any clue as to the pathogenesis of primary systemic amyloidosis.

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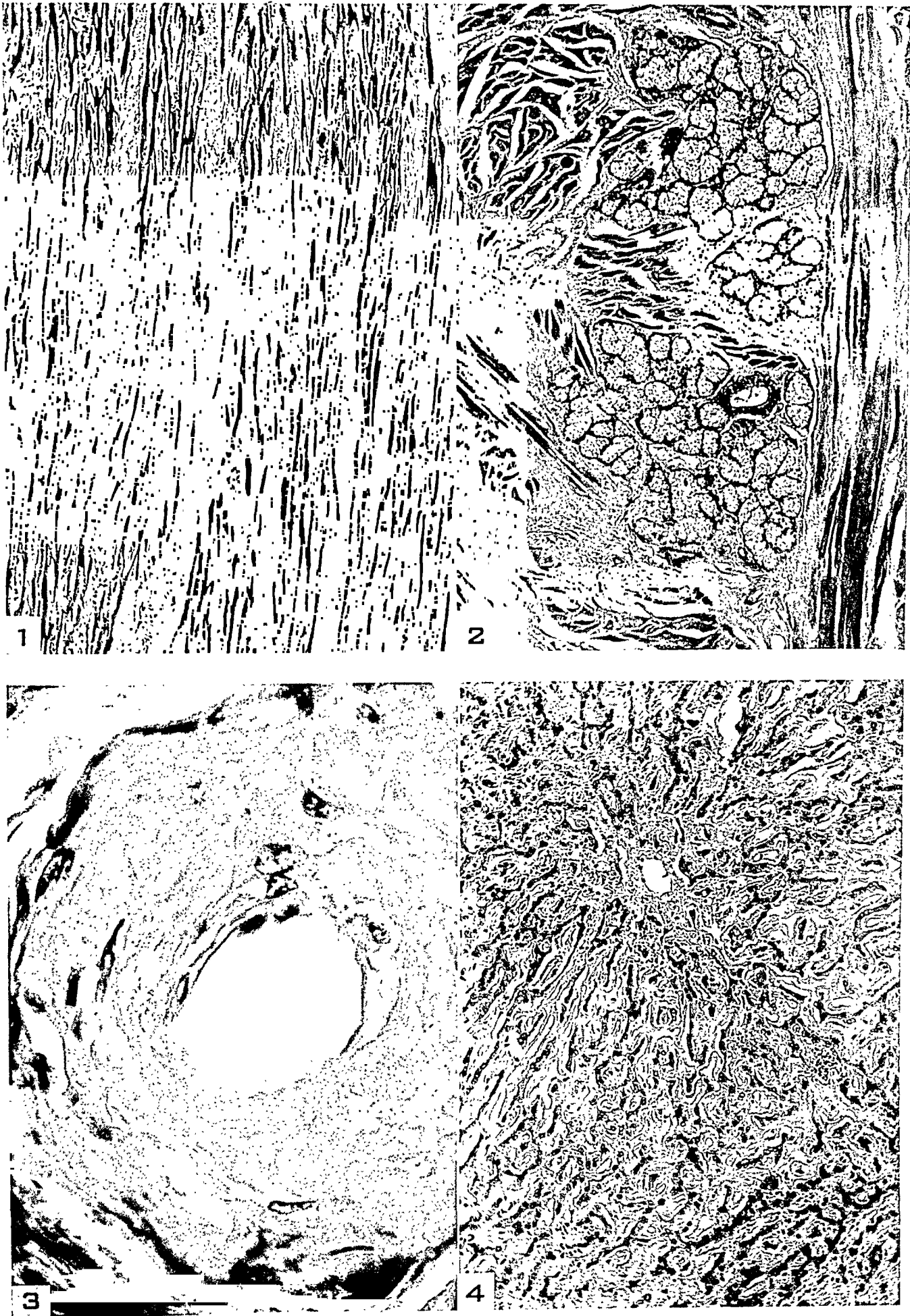
[ Illustrations follow ]

## DESCRIPTION OF PLATES

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### PLATE 15

- FIG. 1. Case 6. Heart muscle, showing diffuse infiltration with amyloid and resultant atrophy of muscle fibers. Hematoxylin and eosin stain.  $\times 55$ .
- FIG. 2. Case 6. Tongue, showing the paler staining amyloid between the mucous glands and the muscle bundles. Hematoxylin and eosin stain.  $\times 55$ .
- FIG. 3. Arteriole, showing amyloid infiltration of the media with separation of the smooth muscle cells. Hematoxylin and eosin stain.  $\times 675$ .
- FIG. 4. Hepatic lobule, showing marked atrophy due to amyloid infiltration. Hematoxylin and eosin stain.  $\times 85$ .



Dahlin

Primary Amyloidosis

# PLATE 16

FIG. 5. Spleen, showing diffuse infiltration with amyloid. Hematoxylin and eosin stain.  $\times 55$ .

FIG. 6. Lung. Dark areas represent amyloid. Photographed using B and H Wratten filter. Methyl violet stain.  $\times 60$ .

FIG. 7. Lymph node markedly infiltrated. Amyloid surrounds the perinodal fat cells. Hematoxylin and eosin stain.  $\times 110$ .

FIG. 8. Thyroid with moderate amyloid infiltration between acini. Hematoxylin and eosin stain.  $\times 130$ .







# THE TOPOGRAPHY OF CHRONIC GASTRITIS IN OTHERWISE NORMAL STOMACHS \*

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Most observers are agreed that the changes in the gastric mucosa which, individually and together, constitute so-called chronic gastritis are frequently encountered in adults (Hillenbrand,<sup>1</sup> Faber,<sup>2</sup> Guiss and Stewart,<sup>3</sup> Hebbel<sup>4</sup>). While it is recognized that such deviations from the normal may be from focal to diffuse in distribution, determinations of the incidence of gastritis in otherwise normal stomachs have referred largely to the qualitative features of the process and there is uncertainty as to the frequency with which varying distributions of the changes may be found. That varying distributions do exist was emphasized by Hillenbrand in a detailed study of 21 stomachs from patients over 35 years of age. As part of a study of the relationship between chronic gastritis and gastric cancer it seemed desirable to have more information concerning the topography of gastritis in stomachs otherwise without disease. Gastritis, commonly found with cancer of the stomach, may vary in its extent (Hebbel) and the significance of that variation can be interpreted only in relation to similar processes found in stomachs without cancer. This paper presents the results of a survey of a group of stomachs with reference to the distribution of the gastritic changes encountered.

## MATERIALS AND METHODS

The material consisted of stomachs obtained at autopsy from persons of both sexes and all ages in whom death resulted from a wide variety of causes and whose past histories recorded no gastro-intestinal complaints. Cases of pernicious anemia were excluded. The material was otherwise selected only by the exclusion of specimens with ulcer, scar, or tumor in either the stomach or duodenum and by further exclusion, after microscopic examination, of those specimens with obscuring post-mortem changes.

Strips of mucosa were removed from the entire lesser and greater curvatures of each specimen and made into rolls of convenient size from which blocks were cut and embedded in paraffin. Sections were prepared in the usual manner. By this means the entire length of the

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stomach on each curvature was examined. From about half of the specimens similar preparations were made from mid-anterior and mid-posterior walls. It had been anticipated that differences might exist, but the findings were so constantly similar to those of the curvatures that preparation of these additional sections was discontinued.

The sections were searched for deviation from the usually accepted normal structure. The several features included in the picture of chronic gastritis are reasonably well established and have been dealt with in many reports (Konjetzny,<sup>5</sup> Hillenbrand,<sup>1</sup> Faber,<sup>2</sup> Magnus,<sup>6</sup> Guiss and Stewart,<sup>3</sup> and many others). Of these, only the following have been included in the accompanying tables (I to VII): Cellular infiltration, lymph follicles, atrophy, intestinal metaplasia, pseudopyloric glands, cystic glands, and erosions. Other accompanying features such as fibrosis, thickening of the muscularis mucosae, Russell's corpuscles, and heterotopic glands have been omitted. To indicate roughly the relative severity or extent, each of the changes, when present, was graded from 1 to 3.

Infiltration refers to the free cells, chiefly lymphocytes, in the stroma. A normal number is not established but there is general agreement (Hillenbrand,<sup>1</sup> Konjetzny,<sup>5</sup> Kalima<sup>7</sup>) that the diagnosis of chronic gastritis should seldom rest on cellular infiltration alone. In this material, infiltration was graded as follows: If free cells were scant and inconspicuous, they were considered to be absent. Grade 1 infiltration indicates a mild degree which was arbitrarily considered within normal limits. Grade 2 indicates moderate, and grade 3 severe, infiltration. There are no sharp divisions between these categories. Few would question that severe infiltration is abnormal and few would insist that a mild degree of infiltration is significant, but there may be difference of opinion as to the proper designation of intermediate degrees. For the purposes of this paper, infiltration in excess of grade 1 has been considered abnormal. In the main, excess infiltration was found in conjunction with other changes and was, with few exceptions, uniform throughout the segment considered. In a few specimens, excess infiltration alone was present and these were kept in a separate category. That such specimens deviate from the ideal normal is certain, but that this probably reversible change is as significant as, or of necessity related to, parenchymal alterations may be questioned.

Lymphoid aggregates, with or without reaction centers, were graded 1 to 3 in the following manner: Grade 1 indicates 1 to 5 nodules, grade 2 indicates 6 to 12 nodules, and grade 3 indicates more than 12 nodules

for the segment of mucosa concerned. No attempt has been made to make antrum and body more comparable in this respect by correcting for the greater length of the strips of body mucosa examined. A normal number has not been established. Konjetzny<sup>5</sup> considered lymphoid follicles to be rare under normal conditions, while Hillenbrand<sup>1</sup> frequently found them in the absence of other changes. In this material, no specimen has been considered abnormal on the basis of the number of lymph nodules alone. Few were encountered in the absence of other changes.

Atrophy, referring to the loss of normal glandular structure, with or without actual thinning of the mucosa, was graded as follows on the basis of distribution and not of severity in any area: Grade 1 refers to one or two foci of microscopic size. Grade 2 refers to several or more foci or small patches. The largest isolated patches encountered rarely exceeded about 5 mm. and larger patches were associated with diffuse changes. Grade 3 refers to atrophy of greater or less degree uniformly involving a whole segment. While the severity of the process varied, it may be noted that the majority of examples of diffuse atrophy were of moderate degree.

Metaplasia refers to the presence of epithelium, similar to that of the small intestine, in crypts and glands. Grade 1 refers to one or two focal areas of microscopic size. Grade 2 refers to several such focal or slightly larger areas. Grade 3 refers to many patchy areas or uniform involvement. The more severe degrees parallel atrophy.

Cysts, referring to cystic changes in the glands, were graded as follows: Grade 1 indicates one or two cystic glands, grade 2 indicates several cystic foci, and grade 3 indicates numerous cysts.

Erosions were rare in this material and those encountered were all healed. They were graded as follows: Grade 1 indicates an isolated erosion, grade 2 indicates several, and grade 3 indicates numerous erosions.

Pseudopyloric glands were graded as follows: Grade 1 refers to one to several focal areas of microscopic size, grade 2 refers to moderate numbers, and grade 3 refers to numerous glands of this type. In the main this feature is observed in association with other changes but may exist alone.

With the exceptions noted in respect to infiltration and lymph follicles, the presence of any of the above changes placed a specimen in the abnormal group. The findings are assembled in Tables I to VII. Since there were no differences related to sex, the sex incidence of the

several changes has been omitted. With some tabulations, totals for the first and second 5 decades of life as well as for the entire series have been given to emphasize the predominance of the changes in the older age groups.

#### PRESENTATION OF DATA

Table I shows the graded findings in each of the several categories for the lesser curvature of the antrum. Infiltration was absent in 20 and present in 77 specimens (44, grade 1; 29, grade 2; 4, grade 3). Follicles were absent in 31 specimens and were present in 66 (36, grade 1; 26, grade 2; 4, grade 3). Atrophy was absent in 61 specimens and was present in 36 (9, grade 1; 16, grade 2; 11, grade 3). Metaplasia was absent in 62 specimens and present in 35 (20, grade 1; 13, grade 2; 2, grade 3). Cysts were absent in 75 specimens and present in 22 (17, grade 1; 5, grade 2). Healed erosions, all grade 1, were present in 12 specimens.

Table II shows, in similar manner, the findings on the greater curvature of the antrum. Infiltration was absent in 21 specimens and was present in 76 (50, grade 1; 23, grade 2; 3, grade 3). Follicles were absent in 34 specimens and present in 63 (45, grade 1; 17, grade 2; 1, grade 3). Atrophy was not found in 71 specimens and was present in 26 (7, grade 1; 14, grade 2; 5, grade 3). Metaplasia was absent in 71 specimens and present in 26 (16, grade 1; 9, grade 2; 1, grade 3). Cysts were absent in 83 specimens and present in 14 (9, grade 1; 5, grade 2). Erosions were absent in 83 specimens and present in 14 (13, grade 1; 1, grade 2).

Table III shows the graded findings for the mucosa of the lesser curvature of the body. Infiltration was absent in 29 specimens and present in 68 (37, grade 1; 26, grade 2; 5, grade 3). Follicles were not present in 33 specimens and were found in 64 (31, grade 1; 30, grade 2; 3, grade 3). Atrophy was absent in 72 specimens and present in 25 (6, grade 1; 5, grade 2; 14, grade 3). Metaplasia was absent in 79 specimens and present in 18 (7, grade 1; 11, grade 2). Pseudopyloric glands were absent in 81 specimens and present in 16 (3, grade 1; 12, grade 2; 1, grade 3). Cysts were absent in 87 specimens and present in 10 (8, grade 1; 2, grade 2). Only one specimen showed a healed erosion.

Table IV shows the findings on the greater curvature of the body. Infiltration was absent from 33 specimens and present in 64 (37, grade 1; 22, grade 2; 5, grade 3). Follicles were absent from 33 specimens and were present in 64 (32, grade 1; 29, grade 2; 3, grade 3). Atrophy was absent in 74 specimens and was present in 23 (10, grade 1; 2, grade

TABLE I  
*Findings, as Graded, on Lesser Curvature of Antrum*

Age groups	No.	Infiltration			Follicles			Atrophy			Metaplasia			Cysts			Erosions				
		0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
I-10	6	5	1			5	1			6	6			6			6				
11-20	4	3	1			3	1			3	3	1		4			3	1			
21-30	11	4	4	3		6	2			11	10	1		10	1		9	2			
31-40	9	4	3	2		4	2	3		6	8	1		9			8	1			
41-50	14	2	8	4		3	6	5		8	8	4	2		3	2	13	1			
51-60	11	7	2	2		4	4	2	1	6	7	2	2	7	4		10	1			
61-70	21	1	9	11		4	11	3	3	11	8	6	5	15	5	1	19	2			
71-80	16	7	7	2		2	5	9		8	10	3	3	11	3	2	13	3			
81-90	4	1	3			4	4			1	1	2	1	3	1		3	1			
91-100	1	1						1		1	1			1			1				
Totals	97	20	44	29	4	31	36	26	4	61	62	20	13	75	17	5	85	12			

TABLE II  
*Findings, as Graded, on Greater Curvature of Antrum*

Age groups	No.	Infiltration			Follicles			Atrophy			Metaplasia			Cysts			Erosions				
		0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
I-10	6	5	1			4	2			6	6			6			6				
11-20	4	3	1			3	1		1	3	3	1		4			3	1			
21-30	11	5	4	2		7	2	2		10	10	1		10	1		10	1			
31-40	9	5	3	1		6	3		1	7	8	1		9			8	1			
41-50	14	2	11	1		3	9	2		12	12	2		12	1	1	11	3			
51-60	11	8	2	1		5	3	2	1	9	9	2		7	4		10	1			
61-70	21	1	11	9		4	12	5		12	13	2	5	18	2	1	18	2	1		
71-80	16	7	7	2		2	8	6		10	8	5	3	14	2	2	14	2	2		
81-90	4	3	1			4	4			1	1	2	1	2	1		2	2			
91-100	1	1				1	1			1	1			1			1				
Totals	97	21	50	23	3	34	45	17	1	71	71	16	9	83	9	5	83	13	1		

TABLE III

Findings, as Graded, on Lesser Curvature of Body

TABLE III																						
Findings, as Graded, on Lesser Curvature of Body																						
Age groups	No.	Infiltration			Follicles			Atrophy			Metaplasia			Pseudopyloric glands			Cysts			Erosions		
		0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	
1-10	6	6	2	1	1	6	4	10	9	11	6	6	6	6	6	6	6	6	6	6	6	
11-20	4	2	2	2	1	2	4	4	1	4	4	4	4	4	4	4	4	4	4	4	4	
21-30	11	6	3	2	1	2	7	10	9	11	11	11	11	11	11	11	11	11	11	11	11	
31-40	9	2	2	2	1	2	2	9	1	9	9	9	9	9	9	9	9	9	9	9	9	
41-50	14	5	6	4	1	5	5	12	1	12	12	12	12	12	12	12	12	12	12	12	12	
51-60	11	1	5	4	2	7	1	7	1	7	7	7	7	7	7	7	7	7	7	7	7	
61-70	21	2	10	7	2	2	2	11	2	13	13	13	13	13	13	13	13	13	13	13	13	
71-80	16	2	7	5	2	5	3	12	2	2	2	2	2	2	2	2	2	2	2	2	2	
81-90	4	1				1		1		1	1	1	1	1	1	1	1	1	1	1	1	
TOTAL	97	29	37	26	5	33	31	72	6	5	14	79	7	11	81	3	12	1	87	8	2	

TABLE IV

Findings, as Graded, on Greater Curvature of Body

Totals		97	29	37	26	5	TABLE IV Findings, as Graded, on Greater Curvature of Body												Erosions										
Age groups	No.	Infiltration				Follicles				Atrophy				Metaplasia				Pseudopyloric glands				Cysts				Erosions			
		0 1 2 3				0 1 2 3				0 1 2 3				0 1 2 3				0 1 2 3				0 1 2 3				0 1 2 3			
		0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
1-10	6	6				4	2			6				6				6				6				6			
11-20	4	4				3	1			4				4				4				4				4			
21-30	11	6	2	1		6	2	2		10				10	1			11				11				11			
31-40	9	6	2	1		5	2	2		9				9				9				9				9			
41-50	14	3	8	3		1	5	4	1	13				13	1			13				13				14			
51-60	11	2	5	3		5	1	5	4	13				9	1			10				11				11			
61-70	21	2	11	7	2	5	8	7	1	16				16	2	3		17				17				20			
71-80	16	4	5	5	2	4	1	6	5	13				13	1			12				15				15			
81-90	4	1				1				2				2				2				2				4			
91-100	1									1				1				1				1				1			
Totals	97	33	37	22	5	33	32	29	3	74	10	2	11	83	6	7	1	85	2	9	1	87	8	2		95	2		

2; 11, grade 3). Metaplasia was found in 14 specimens (6, grade 1; 7, grade 2; 1, grade 3) and was absent from 83. Pseudopyloric glands were absent in 85 specimens and were present in 12 (2, grade 1; 9, grade 2; 1, grade 3). Cysts were encountered in 10 specimens (8, grade 1; 2, grade 2) and were absent in 87 specimens. Only 2 specimens showed healed erosions (both grade 1).

Table V consolidates the findings in the antral mucosa. Thirty-three specimens (34 per cent) showed no change on either curvature. In 19 specimens (19.6 per cent) the changes were confined to the lesser curvature and, of these, the changes were focal in 15 and patchy in 4. In 2 specimens (2.1 per cent) there were isolated focal lesions on the greater curvature only. Ten specimens (10.3 per cent) showed changes over the whole lesser curvature with associated focal changes on the greater curvature. Of these, 2 showed only excess infiltrate. No specimens showed diffuse changes on the greater curvature alone. Both curvatures were similarly involved in 33 specimens (34 per cent). Of these, both curvatures showed focal lesions 12 times and small patchy lesions 9 times. The remaining 12 specimens showed diffuse changes: 4 showed excess infiltrate only, and 8 (8.2 per cent of entire series) showed diffuse parenchymal changes (chiefly atrophy and metaplasia). It is to be noted that 7 of these 8 specimens were among the 53 from persons over 50 years of age (an incidence of 13 per cent) and but one was among the 44 specimens from persons in the earlier decades (an incidence of 2.3 per cent).

Table VI consolidates the findings with reference to their distribution in the body mucosa. In 49 specimens (50.5 per cent) both curvatures were unchanged. Eight specimens (8.2 per cent) showed changes on the lesser curvature only. Three of these showed only excessive infiltrate, 3 showed focal lesions, and 2 showed patchy lesions. Seven specimens (7.2 per cent) showed changes on the greater curvature only. In one of these there was a moderate infiltrate only, while in 6 there were isolated focal lesions. In 3 specimens (3.1 per cent) there were diffuse changes on the lesser curvature (1 with excessive infiltrate only, 2 with parenchymal changes) and only focal lesions on the greater curvature. In one specimen (1 per cent) excessive infiltrate on the greater curvature was associated with focal changes on the lesser curvature. (In this case there was actually a similar infiltrate on the lesser curvature, but the presence of parenchymal change placed it in the distinctly abnormal category.) Twenty-nine specimens (29.9 per cent) showed comparable changes on both curvatures. Eight of these showed isolated focal or patchy lesions and 21 (7 with excessive infil-







trate only) showed diffuse changes. Of the specimens with diffuse changes, 6 were from the 44 persons in the first 5 decades and 15 were from the 53 in the second 5 decades of life. Considering only the 14 specimens with parenchymal changes (14.4 per cent of the entire series), 3 were from the former group (an incidence of 6.8 per cent) and 11 were from the latter group (an incidence of 20.8 per cent).

TABLE VII  
*Summary of Findings in Antrum and Body Combined*

Age	Males	Females	Total no. specimens	Antrum and body both normal	Body normal, antrum abnormal		Antrum normal, body abnormal		Both antrum and body abnormal			
					Focal, patchy, etc.	Diffuse	Focal, patchy, etc.	Diffuse	Focal, patchy, etc. in both	Diffuse in antrum, focal etc. in body	Focal etc. in antrum, diffuse in body	Diffuse both antrum and body
1-10	5	1	6	6								
11-20	3	1	4	1	2	1						
21-30	4	7	11	5	2		1		1	1		1
31-40	4	5	9	3	3		1				2	
41-50	8	6	14	5	4				2		3	
51-60	9	2	11	1	5		1		1	1	1	1
61-70	13	8	21	3	2		1		8	2	4	1
71-80	11	5	16	3	1	2			4	1	5	
81-90	2	2	4				1			1	2	
91-100	1	0	1				1					
Total	60	37	97	27	19	3	5	1	16	6	17	3
First 5 decades	24	20	44	20	11	1	2	0	3	1	5	1
Second 5 decades	36	17	53	7	8	2	3	1	13	5	12	2

The distribution of the changes for the whole stomach is shown in Table VII. Changes less than diffuse are here combined and the variations, from focal lesions to alterations over the whole of a single curvature, thereby lose identity. These specimens, however, are all quite sharply set apart from those with uniformly diffuse changes. Reference is made to the variations below.

Both antrum and body were free of change in 27 specimens (27.8 per cent). Twenty of these were from persons in the first 5 decades and 7 were from those in the second 5 decades.

Twenty-two specimens (22.7 per cent) showed changes in the antrum and a normal body mucosa. In 3 of this group the antrum showed diffuse changes (2 with parenchymal changes, one with excessive infil-

trate only). The remaining 19 showed less than diffuse changes and, of these, 17 showed focal or patchy lesions on one or both curvatures and 2 showed only excessive infiltrate on one curvature. As may be seen in Table VII, specimens in this group fall throughout the several decades and were proportionately somewhat more frequent in the earlier than in the later decades. In quantitative terms it is doubtful that any of the 19 should be considered significantly abnormal.

In 6 specimens (6.2 per cent) a normal antrum was associated with changes in the body mucosa. In 4 of these the body mucosa showed isolated focal lesions, one showed only an excessive infiltrate confined to the lesser curvature, and one showed diffuse parenchymal changes. In this group only one specimen, the last, can be considered quantitatively abnormal.

Both antrum and body showed changes of some degree in 42 specimens (43.3 per cent) and, as shown in Table VII, there was considerable variation in this group. In 16 of these the changes were less than diffuse in each of the two mucosal divisions. Seven of the 16 showed changes (2 with only excessive infiltrate) in the entire lesser curvature of antrum or body and the remainder showed focal or small patchy lesions. Six specimens showed diffuse changes in the antrum (one with excessive infiltrate only) and but focal lesions in the body. Seventeen specimens showed less than diffuse changes in the antrum and diffuse changes in the body. Here the body mucosa showed only excessive infiltrate 4 times and parenchymal changes 13 times. Twelve of the 17 were from persons over 50 years of age. In but 3 specimens were the changes diffuse in both antrum and body. One showed only excessive infiltrate in both regions, one showed excessive infiltrate in the antrum and parenchymal changes in the body, and one showed diffuse parenchymal changes in both.

#### COMMENT

Wide variation has been noted in the distribution of gastritic changes, ranging from focal to diffuse processes involving all of the antrum or body, but rarely both. There is no uniformity in the pattern of involvement of the antrum and body of the same stomach and these divisions must, in the main, be considered separately. These marked variations in distribution emphasize the necessity of designating the source of gastric material subjected to microscopic examination. It is clear that the presence of lesions on the lesser curvature of the antrum or body is not necessarily indicative of diffuse changes. On the other hand, extensive changes were encountered on the greater curvature only

as part of a diffuse process in the segment concerned. The uniformity of change from one area to another under these circumstances has been mentioned. It would appear, then, that changes, in excess of a focal lesion, encountered in a section of reasonable size from the greater curvature of the antrum or body well reflect the condition of the whole segment in so far as diffuse processes are concerned. The inference may be drawn, however, only in the case of stomachs otherwise free of disease.

While 27 specimens were free of change, many of those in which changes were found presented only isolated focal or patchy lesions. It seems quite certain that, if enough sections were prepared, few of the 27, at least among adults, would be entirely free of change. Consequently, a number of those tabulated as abnormal must be considered free of significant change. Where, short of diffuse involvement, to draw a line between normal and abnormal on the basis of quantitative change must be arbitrarily determined and remains uncertain. In this material there are no specimens which serve fully to bridge the gap between those showing focal or patchy lesions and those with diffuse processes. A somewhat intermediate position is taken by those specimens which showed changes along the entire lesser curvature with but focal or no changes on the greater curvature. This distribution was more frequently encountered in the antrum. How far on the anterior and posterior walls such a process may extend is not certain. The findings in this material suggest that, particularly in the body, the process is limited to the immediate vicinity of the curvature, but the number of pertinent cases is small and the impression could well be modified by a larger material. In those specimens which showed diffuse changes the process was quite uniform throughout and they were, consequently, sharply set apart from those with lesser degrees of abnormality. The uniformity of the process from one area to another suggests simultaneous involvement of the whole in its evolution. Here again the number of pertinent cases is too small to permit exclusion of spreading changes.

As in a previously reported series,<sup>4</sup> there is nothing in the available information concerning the patients represented which gives any clue to the origin or causes of the changes encountered. The only constant association seems to be that of advancing age. There is no necessity of believing that all of the changes encountered are causally related. That one may be observing qualitatively similar end-stages of reactions to a variety of causes seems likely. The striking separation between focal and diffuse processes as observed here may be a reflection of such differences.

It appears that the findings described are at least roughly indicative of the expected changes in the stomachs of individuals free of manifest gastric disease. The incidences recorded of the several degrees of change would no doubt be modified by a larger material. That the data fairly reflect the autopsy experience of this laboratory seems reasonable and is supported by collateral evidence. The 97 specimens on which this study was based were selected from a much larger group of similar specimens because of freedom from post-mortem change in the sections examined. It has been noted that those with diffuse changes presented a similar pattern throughout and, consequently, uniform changes in a relatively small area of the greater curvature fairly well reflect the condition of the whole in respect to the presence or absence of diffuse processes. Though unsuitable for inclusion in this series, those specimens which showed partial post-mortem changes in the rolls or those from which blocks only were available could be used for the determination of the incidence of diffuse changes. On this basis, diffuse gastritis has about the same frequency as noted above. Furthermore, allowing for the differences in technic of examination, the findings in this study compare rather favorably with those reported by me previously<sup>4</sup> on a similar material.

#### SUMMARY

Gastritic lesions were found at autopsy in 70 (72 per cent) of 97 stomachs of persons free from manifest gastric disease. Of the 27 stomachs which showed no change, 20 were from the group of 44 persons less than 51 years of age and 7 were from the group of 53 persons over 50 years of age. There was no uniformity of involvement between antrum and body and the changes in each segment varied widely from isolated foci to diffuse alterations. Each segment is best considered separately. Many of the lesions encountered are not quantitatively significant but where, short of a diffuse gastritis, to draw a line between normal and abnormal on the basis of quantitative change is uncertain. The antrum was abnormal in some degree in 64 specimens (66 per cent of the total, 50 per cent of those from persons less than 51 years, 79 per cent of those from persons over 50 years of age). The lesions in the younger group were largely focal. Diffuse parenchymal changes were found in 8 specimens (8.2 per cent of the total), or in 1 (2.3 per cent) of those from persons under 51 years and in 7 (13 per cent) of those from persons over 50 years of age. The body mucosa was abnormal in some degree in 48 specimens (49.5 per cent of the total, 27 per cent of those from persons less than 51 years, 68 per cent of those from persons over 50 years of age). Diffuse parenchymal changes were found in 14 specimens (14.4 per cent of the total), occurring in 3 (6.8

per cent) from persons less than 51 years and in 11 (20.8 per cent) from persons over 50 years of age.

### CONCLUSIONS

In otherwise normal stomachs, gastritic lesions are common. They predominate in the older decades, do not uniformly involve antrum and body, and within either area range from focal to diffuse in distribution.

Diffuse gastritis with parenchymal changes was found at autopsy in the antrum in 8 per cent and in the body in 14 per cent of a series of 97 stomachs of persons free of manifest gastric disease.

The gastritic changes observed in conjunction with other gastric lesions must be interpreted in the light of associated findings.

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### DESCRIPTION OF PLATES

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#### PLATE 17

- FIG. 1. Normal antral mucosa. Hematoxylin and eosin stain.  $\times 65$ .
- FIG. 2. Antral mucosa with moderately heavy lymphocytic infiltration which splits the muscularis mucosae. Hematoxylin and eosin stain.  $\times 65$ .
- FIG. 3. Focus of intestinal metaplasia in antral mucosa adjacent to lymph follicle. Hematoxylin and eosin stain.  $\times 65$ .
- FIG. 4. Antral mucosa showing complete atrophy of glands and scant lymphocytic infiltration. Remaining crypts show epithelium of the intestinal type. Hematoxylin and eosin stain.  $\times 65$ .





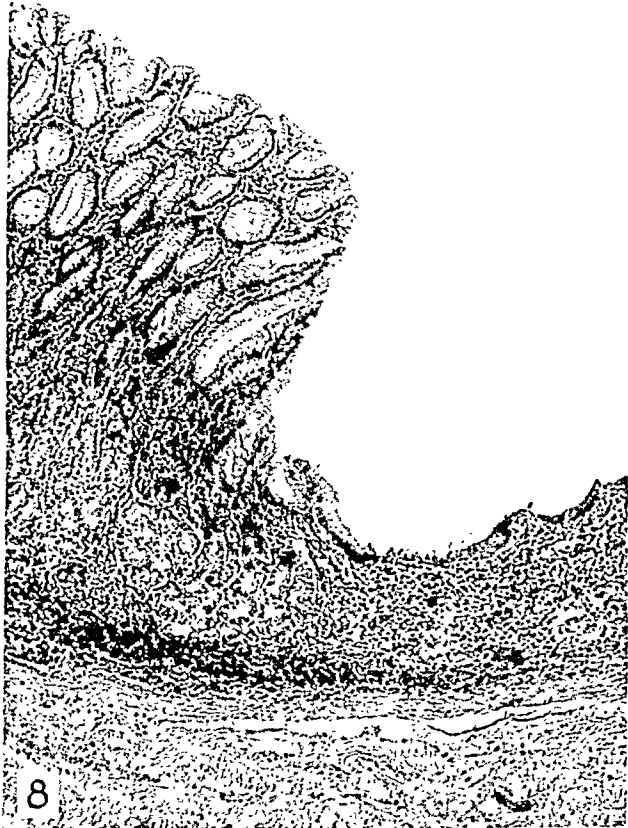
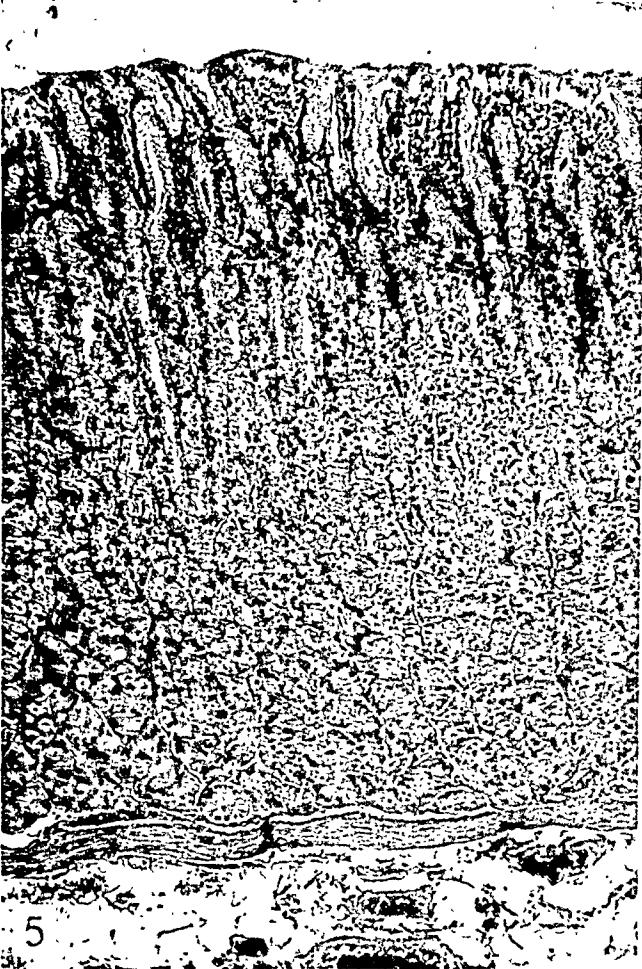
PLATE 18

FIG. 5. Normal body mucosa with mild interfoveolar lymphocytic infiltration. Hematoxylin and eosin stain.  $\times 65$ .

FIG. 6. Body mucosa showing heavy lymphocytic infiltration in superficial half and mild infiltration below. The crypts are deepened and the functional parts of the gland tubes correspondingly reduced. Hematoxylin and eosin stain.  $\times 65$ .

FIG. 7. Body mucosa showing complete loss of normal glandular structure and presence of numerous pseudopyloric glands. Hematoxylin and eosin stain.  $\times 65$ .

FIG. 8. Healed erosion in body mucosa. Adjacent glands partly replaced by pseudopyloric glands. Hematoxylin and eosin stain.  $\times 65$ .





# DESTRUCTION OF CARTILAGE CELLS IN THE NEWBORN RAT BY BRIEF REFRIGERATION, WITH CONSEQUENT SKELETAL DEFORMITIES \*

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Following exposure to cold surfaces, remarkable distortion and retardation in growth of the tail and limbs of newborn rats have been observed (Scow, 1944). The deformities encountered took the form of short and wedge-shaped vertebrae producing marked angulation of the tail, bizarre curvature of the shaft, and rotation of the joint surfaces of the long bones, as well as shortening of the metatarsal, metacarpal, and phalangeal bones (Figs. 1 and 2). These phenomena followed the employment of refrigeration anesthesia in the course of which the rats were placed for 15 minutes in a glass beaker with the internal temperature reduced to  $-5^{\circ}$  to  $-10^{\circ}$  C. by partial submergence in a mixture of ice cubes and brine (Scow and Simpson, 1945). The resulting anesthesia, attended by temporary cessation of respiratory movements and marked reduction of cardiac rate, lasted from 8 to 15 minutes. Skeletal deformities were first noted several weeks later in about one-fifth of the 200 animals anesthetized. Further investigation demonstrated that deformities occurred only in those parts which had come in contact with the cold surfaces of the beaker, and were more severe following either a longer period of exposure or exposure to colder surfaces. These skeletal deformities usually were not associated with gross disturbances of general growth or of adjacent soft tissues, except in a few instances in which the exposure was sufficient to produce gangrene. The animals exhibiting skeletal deformities showed no other alterations in growth or differentiation.

The purpose of the following experiments was to determine the cause of the retardation in growth and differentiation affecting the skeletal elements. The tail of the newborn rat was employed because of its accessibility for exposure to cold, the ease with which the earlier stages of development of bone could be followed, and the readiness with which the deformities could be observed and recorded by roentgenograms.

## EXPERIMENTAL PROCEDURE

A total of 55 newborn rats of both sexes of the Long-Evans strain were employed. After exposure to cold, the animals were returned to their mothers for nursing and were weaned on the 21st day of life. The parents did not hesitate to receive them. The mortality following experimental refrigeration was exceedingly small.

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The walls of a glass beaker submerged in a mixture of ice cubes and brine are of a temperature sufficiently low ( $-10^{\circ}$  C.) to produce deformities, but the time exposure is unduly long and the experiments difficult to control. It was found later that when the newborn rat was placed on the surface of a piece of ice sprinkled with salt, similar results could be obtained. The method proved difficult to standardize, however, and a period of 10 to 15 minutes was required to produce freezing of the tail.

The method adopted, therefore, was to place the tail of the 1-day-old rat in contact with the surface of the freezing plate of a freezing microtome. The plate was frosted "white" prior to the exposure and the tail was then gently held against it for 15 to 20 seconds until it became white. After exposure, the rat was placed in a moderately warm place (6 to 8 inches below a 40 watt electric lamp) for 1 hour before being returned to the mother.

The animals were sacrificed at varying intervals, from  $\frac{1}{2}$  hour to 38 days after exposure. In those permitted to survive for periods in excess of 10 days, the resulting deformities were observed and recorded by roentgenography. Calcification of the vertebral segments in rats younger than 10 days is inadequate to permit roentgenographic study.

For histologic study, the tail, skinned except for the distal 1 cm., was fixed in 10 per cent neutralized formalin, decalcified in 5 per cent nitric acid, and infiltrated and embedded in celloidin. The tissue was sectioned at 8 to 10  $\mu$  and the sections were stained with hematoxylin and eosin.

## OBSERVATIONS

### *Macroscopic Study*

The tail of the newborn rat \* became white after being held for 15 to 20 seconds against the freezing plate. The "white stage" lasted 20 to 30 seconds and was followed almost immediately by a reddening of the whole tail which persisted for several hours. The hyperemia usually subsided by the twelfth hour, unless gangrene supervened, and was not accompanied by gross edema. Retardation of growth was first evident 3 to 4 days after freezing. Angulation and deformity of the tail usually were apparent after the fifth day. The deformity increased progressively as the animal became older. It is very important to note that, except in a few cases, there was no evidence of permanent damage to the soft tissue. In the older animals, the external appearance of the tail usually was indistinguishable from that of the normal, with the exception of the decrease in length or the angulation, or both. In those

\* Average size of tail of these newborn rats: length, 1.5 to 1.8 cm.; thickness (at base of tail), 1.7 mm.

animals developing gangrene, the first evidence of its appearance was the maintenance of the hyperemia followed by a chocolate discoloration of the skin. The skin was then easily shed on the second or third day; in some instances, the end of the tail dried up and dropped off spontaneously on the eighth to tenth day.

Roentgenograms taken on the 10th day after freezing demonstrated irregular areas of failure of calcification in the vertebrae.\* The calcified area of some of the affected vertebrae was wedge-shaped instead of being rectangular. In other vertebrae the calcified centra were shorter than expected, or had irregular shapes, or exhibited irregularities at their distal or proximal surfaces. The wedge-shaped vertebrae invariably were associated with angulation of the tail, which increased in degree and extent with age (Fig. 1). The epiphyseal line of the affected vertebrae in the 38-day-old animals was irregular in outline and in others the epiphyses occupied abnormal positions (Fig. 2).

### *Microscopic Study*

The normal caudal vertebra of the newborn rat consists of a mass of hyaline cartilage, bounded by perichondrium peripherally and by an intervertebral disk (consisting of the nucleus pulposus encircled by the annulus fibrosus) on its distal and proximal surfaces (Fig. 3). The cartilage exhibits three general zones of development: (1) At either end, irregular isogenous groups of typical hyaline cartilage cells, succeeded by (2) a zone of flattened cells in columnar arrangement, and finally (3) a central area of hypertrophic or vesicular cells (which are undergoing or are soon to undergo calcification) (Maximow and Bloom, 1942). There is no endochondral or periosteal ossification and very little, if any, calcification of the cartilage at birth. The skin is firmly attached to the underlying vertebrae and attendant ligaments by means of a dense, fibrous network. The subcutaneous tissues of the tail, therefore, afford little opportunity for collection of fluids and development of edema.

On freezing, destruction of the cartilaginous vertebra varied in extent from a few scattered necrotic cells to necrosis of the entire cartilage (Fig. 4). The extent of damage seemed related to the depth of penetration of cold and could be determined readily by tracing the affected cells centrally from the surface to which cold was applied. Sometimes only the edge of the most lateral parts of the vertebra was damaged; at other times, the area of destruction included a greater portion of the vertebra. The large hypertrophic cartilage cells in the center of the

\* The calcified area in the caudal vertebra of the normal 10-day-old rat consists of the calcified cartilage, the adjacent endochondral bone, and the periosteal bone. At this age, the epiphyseal plate and epiphysis have not been formed.

vertebra, which are the first to be replaced by bone, were the most resistant of all cartilage cells to the freezing process. The area of degeneration nearly always was defined by a curved border concave toward the injured side.

The changes in the cartilage cells, which were found to be permanent and associated with the death of the cells, were seen in the tail vertebrae 30 minutes after exposure to cold (the earliest specimens). The immediate changes consisted of basophilic condensation of the nucleus, loss of the nucleolus and filamentous structure, and eosinophilic granulation of the cytoplasm which was withdrawn from the lacunar wall, leaving a clear space. Four hours after exposure, the shrunken cytoplasm was still more eosinophilic and the nucleus more basophilic. The affected cells were found sparsely scattered throughout otherwise normal-appearing cartilage, or solely occupying large areas of the vertebrae. At later stages the cells showed further shrinkage and the nucleus accepted the eosinophilic stain more readily. In 30-day-old animals, not uncommonly, islands of these eosinophilic ghosts could be seen enclosed within the bone which had now developed. At no time had they initiated any foreign body reaction.

In order to demonstrate further that these alterations were brought about immediately by freezing, epiphyseal cartilage from the knee joints of several three-quarter-term bovine fetuses were studied. The material was obtained and used less than 1 hour after the death of the mother. Cellular changes similar to those found in the tails of rats after freezing were observed in the unfixed tissue immediately after sectioning small pieces of the epiphyseal cartilage with the freezing microtome. These necrotic changes also were observed in small pieces of the cartilage which were frozen for 1 minute, fixed in 10 per cent neutral formalin for 2 days and then sectioned with the freezing microtome. However, cartilage fixed for 2 days in formalin before freezing and sectioning did not show these changes.

The earliest signs of repair were seen 30 hours after freezing, when large areas of the cartilage containing very faint eosinophilic ghost cells were invaded from the periphery by cells of a fibroblastic nature (with basophilic cytoplasm and large basophilic nuclei containing nucleoli). The perichondrium and vertebral borders of the intervertebral disk appeared to be undergoing considerable activity, yet very few mitotic figures were seen. In some of the older specimens, islands of living cartilage cells appeared to have arisen from scattered, surviving cartilage cells (Fig. 7).

An interesting phenomenon was seen in the 20-day specimens in which the vertebrae were wedge-shaped. On the unfrozen side, the bone appeared to be almost normal (*e.g.*, bone with periosteal ossification on the lateral side, and endochondral ossification at each end; Fig. 7) and was separated from the frozen side of the vertebra by a narrow area about one quarter the length of the long side, composed of the "shrunk" necrotic cartilage cells. This necrotic area was attached at both ends to the adjacent nucleus pulposus. The nucleus pulposus was no longer enclosed by the annulus fibrosus and also had become displaced to the shorter side. On the short side of the vertebra—that exposed to freezing—bone, twice as wide and less than one-half as long as that on the unfrozen side, had arisen from the periosteum. No endochondral ossification could be observed on this short side. It would appear that the excessive periosteal bone formation was the result of either stimulation by cold or by the adjacent, destroyed cartilage cells.

The nucleus pulposus in several specimens was displaced laterally to the damaged side, occasionally escaping from the annulus fibrosus. The nucleus pulposus first appeared to move laterally as the uninjured side of the vertebra increased in length (Fig. 6).

One is struck by the selective damage to the tail caused by brief refrigeration, namely, destruction of cartilage with other tissues remaining relatively intact. In some instances, the cartilage cells of the entire vertebra were necrotic, yet there was little damage to the other structures of the tail. The next most susceptible tissue, less frequently injured, was the muscle lying adjacent to the lateral walls of the vertebra. In very few instances, the collagenous fibers of the subcutaneous tissue showed degenerative changes, and least frequently seen was necrosis of the peripheral layer of the epidermal cells. The blood vessels never were found to contain thrombi. The cells of the intervertebral disk (nucleus pulposus and annulus fibrosus), of the tendons of the tail, and of the perichondrium did not seem to be susceptible to cold with such brief exposure.

## DISCUSSION

### *Changes in Cartilage Cells*

The great susceptibility of cartilage to the lethal effects of brief refrigeration is an interesting phenomenon and one which leads to remarkable disturbances of skeletal growth and development in the rapidly growing newborn rat.

Rischpler, in 1900, reported that all tissues which he studied suffered



under the influence of lowered temperatures even when the exposure was for a duration of but 3 to 4 minutes. The damage consisted primarily of an alteration of the cell and its nuclear structure, and it was directly proportional to the duration and degree of cooling. The location and structure of each cellular element also played a rôle in determining the degree of damage. He noted that the epiphyseal cartilage cells and their nuclei in the mouse tail showed signs of damage 20 minutes after freezing by an ether spray.

In extensive study of the effects of freezing on living tissues, Burckhardt, in 1926, reported findings similar to those discussed here. He examined the effect of freezing on the elbow joints of rats at varying ages to evaluate the relative sensitivity of the tissues involved, and to assess their relative regenerative powers. He found epiphyseal cartilage, bone, and articular cartilage of newborn rats to be most sensitive, and observed also the distinct line of demarcation between the injured and uninjured cells within the cartilage, as noted in the present observations. On the day following freezing, he found that the injured cartilage cells stained weakly and, later, disappeared, possessing no regenerative power. Articular cartilage of somewhat older animals, bone tissue (both compact and spongy) of all older groups, bone marrow, and periosteum were found to be next in degree of sensitivity. The developed cartilage of adult or nearly adult rats was considerably less sensitive. Relative to the large blood vessels and the skin, he could infer only that their function remained preserved even after the most severe freezing.

Strandell (1941) found that cartilage and, more specifically the unclosed epiphysis, was especially sensitive to cold.

Haas (1942) reported a microscopic study of the interaction between crystal violet and various human embryonal tissues which were sectioned at 15 to 20  $\mu$  on a freezing microtome. He described a "positive reaction" as consisting of a "microscopic interaction in the presence of nitrous acid between a particulate product derived from crystal violet and an unknown cytoplasmic component which is especially abundant in the region of the cytoplasmic membrane." He found this positive reaction to be specific for certain types of infantile cartilage. The subperichondral cells and cells in the central portion of epiphyseal cartilage gave a positive reaction whereas "the large swollen [cartilage] cells proximal to the deposits of osteoid tissue," the perichondrium, and the neighboring fibrocytes failed to react. All of the noncartilaginous tissues studied failed to give a positive reaction whereas the reac-

tion of adult cartilage was not uniform. It is interesting to note that Haas has found this reaction to be specific for epiphyseal cartilage with the exception of "the large swollen [cartilage] cells proximal to the deposits of osteoid tissue." The latter cells also were found in the rat tails in this study to possess a low sensitivity to freezing in contrast to the other cell types of young cartilage. This "immunity" might be related to some change in the vesicular cells during their degeneration, prior to invasion by the vascular connective tissue in the process of endochondral ossification.

In view of the high sensitivity of cartilage to cold, it is suggested that the "positive reaction" and its specificity for the cartilage cells, as described by Haas, may depend upon a chemical or physical change in some component of the protoplasm of cartilage cells brought about by freezing (on the microtome), during the preparation of specimens for sectioning, which is related to the death of the frozen cartilage cells as observed in this study. Observations of the reaction of cartilage to freezing have been infrequently noted in the literature. Many of the studies carried out on freezing have been made on pathologic specimens from human adults, or on portions of animals in which cartilage (especially epiphyseal or young cartilage active in endochondral ossification) was not present.

### *Skeletal Anomalies*

The subsequent development of skeletal anomalies in the rat tails and limbs which had been exposed to brief freezing on the first day of life has been clearly shown to be the result of destruction of cartilage cells active in endochondral ossification. The degree of the deformity depends upon two main factors: (1) The initial extent of cartilage destruction (*e.g.*, a whole vertebra, or a portion); and (2) the duration of time after the freezing. Since cartilage is the precursor of bone, laying down a "mold" of the skeletal part and later taking active part in the linear growth of the bone at the epiphyseal lines, its destruction obviously will lead to deformities. As an example, in the vertebra of a newborn rat which has sustained damage to the cartilage in its lateral portion, growth at the ends of the diaphysis occurs with an increase in the length of the vertebra on the less damaged side, whereas the other side may partially or completely fail to increase in length because of the dead cartilage cells. The difference in the length of the two sides of the vertebra accompanied by a distortion of the pattern of the normal vertebra will increase progressively. An example is cited of the knee

joint of one rat which was held against the cold surface and sustained lethal damage to cartilage cells on that side of the adjoining two bones.\* By the 38th day, the joint surfaces of these two bones had each revolved more than  $100^{\circ}$ , with an accompanying curvature of the bone shaft.

Löhr, in 1930, reported an isolated case of a 16-year-old child who had frozen one hand with subsequent development of deformity and lack of growth in the little finger, which roentgenographically showed an absence of the epiphyseal line in the distal phalanx. In the hands of 5 adults, similarly exposed, he was unable to find damage in or near the joints.

Burckhardt (1930) also noticed this difference in response to freezing by cartilage in animals of growing age and in those of adult age. He felt that epiphyseal cartilage was sensitive to acute and chronic freezing, and that the damage was irreparable. He produced interference with growth following applications of cold ( $\text{CO}_2$ ) to the elbow of the rat, and observed that the lower end of the humerus (shaft and distal end) remained smaller than is normal. The dead cartilage of the epiphysis either underwent gradual disappearance or remained as a hyaline band.

### *Mechanism of Necrosis*

It has been shown clearly that cartilage cells undergo immediate changes following exposure to cold below the freezing point of water and that these changes—basophilic condensation of the nucleus with loss of its nucleolus and filamentous structure, and an eosinophilic granular condensation of the cytoplasm with a withdrawal of the cytoplasm from the lacunar wall—are associated with the failure of these cells to take their normal active part in the process of endochondral ossification. It is believed that these cells therefore suffer an immediate death. Areas of living cells, which have been shown to take part in the growth of the tail, are seen directly in contact with areas of the affected cells, and the line of demarcation between the two areas is observed to be either straight or concave towards the affected or frozen side. This line probably represents the depth of penetration by the cold.

The mechanisms by which cold brings about tissue destruction can

\* The epiphyseal lines of the tibia and the femur adjacent to the knee joint were each found to have rotated about  $120$  to  $150^{\circ}$  (in relation to the normal position of each) in a 38-day old rat after the knee joint had been held against the freezing microtome plate for 1 minute on the first day of life (Fig. 8). (The roentgenogram of this knee joint is seen in Fig. 2). On the first day of life, the epiphyseal body had not yet appeared according to the roentgenograms; thus the true epiphyseal line was not present. The latter was probably represented by the line of active endochondral ossification. Undoubtedly the epiphyseal line-to-be was injured on one side by freezing with a consequent reduction of increase in length of that side of the bone; and with a relatively uninterrupted growth on the opposite side, the epiphyseal line as well as the articular surface of the bone was rotated as compared to the normal knee joint.

be divided into two general types: *indirect* and *direct*. Much interest has been shown recently in the *indirect* effects of cold, by which a disturbance in the circulation to the part is brought about, with consequent degenerative processes which may lead to gangrene (Adami, 1910; Askanazy, 1913; Smith, Ritchie, and Dawson, 1916; Lake, 1917; Lewis, 1941; Greene, 1943; Friedman, 1945; Lange, Boyd, and Loewe, 1945; Kreyberg, 1946). This has been proposed as the pathogenesis of immersion foot, trench foot, and frostbite. In a large body of tissue, such as the extremities of man, the amount of the tissue damaged is in excess of that which has been penetrated by cold (Lake, 1917). In these cases, there is a latent period between exposure and loss of tissue.

Cold can affect the tissue, also, by its *direct* action upon individual cells (Askanazy, 1913; Smith *et al.*, 1916; Blackwood and Russell, 1943), as occurs in the superficial tissue destroyed by cold, and also in small bodies of tissue which are easily penetrated by cold. In studies of effects of cold upon living tissue, ranging from bacteria through larger plant cells to animal tissues, both normal and malignant, several different processes have been suggested: (1) Cellular rupture (Chandler and Hildreth, 1935); (2) alteration of enzyme systems (Haines, 1937; Safford and Nathanson, 1944); (3) alteration of proteins in protoplasm (Askanazy, 1913; Lake, 1917; Haines, 1937); (4) dehydration by salt disturbances (Rischpler, 1900; Lambert, 1912; Moran, 1929); and (5) acute aseptic inflammation (Brownrigg, 1945; Kreyberg, 1946).

The tail of the newborn rat is so small that penetration of a large portion of the exposed tissue by cold is possible. No evidence of vascular occlusion was seen in the tissues studied. The changes were seen immediately after freezing, much too soon for them to be due to vascular deficiency. They were noted also in portions of cartilage tissue completely removed from the organism as a whole and from the vascular system. Because of these observations, it is felt that the changes are the result of the direct effect of cold upon the tissue. It is difficult to evaluate the various mechanisms listed for the mode of action of the direct effect of cold, but it can be said that the microscopic observation of marked alteration in cytoplasm and nucleus suggests that the cellular retardation observed in this study is the result, at least in part, of an alteration of proteins of the cell.

#### SUMMARY

Cartilage cells in the unossified caudal vertebrae and limbs of newborn rats were found to be highly susceptible to brief refrigeration. The other tissues studied and listed in order of decreasing susceptibility

to cold were muscles, subcutaneous tissue, and skin. The cells of the intervertebral disk, perichondrium, future periosteum, and caudal tendons did not seem susceptible. The effect of cold on osseous tissue was not studied because of the absence of bone in the tail of the newborn rat.

Progressive marked alteration and retardation of growth with bizarre deformities were observed in these skeletal parts, following very brief exposure to cold.

These skeletal changes have been shown to result from necrosis of the cartilage cells which are normally active in lengthening and shaping skeletal parts through endochondral ossification.

The findings presented support the hypothesis that the lethal effects of cold act directly upon these cells, and that the changes include a marked alteration of the proteins in the cytoplasm and nucleus. No thrombi were seen.

The intervertebral disk adjacent to injured caudal vertebrae was observed to be shifted laterally to the frozen side, apparently by the differential growth of the frozen and the unfrozen parts of the vertebra. The nucleus pulposus often escaped from within the annulus fibrosis in these specimens.

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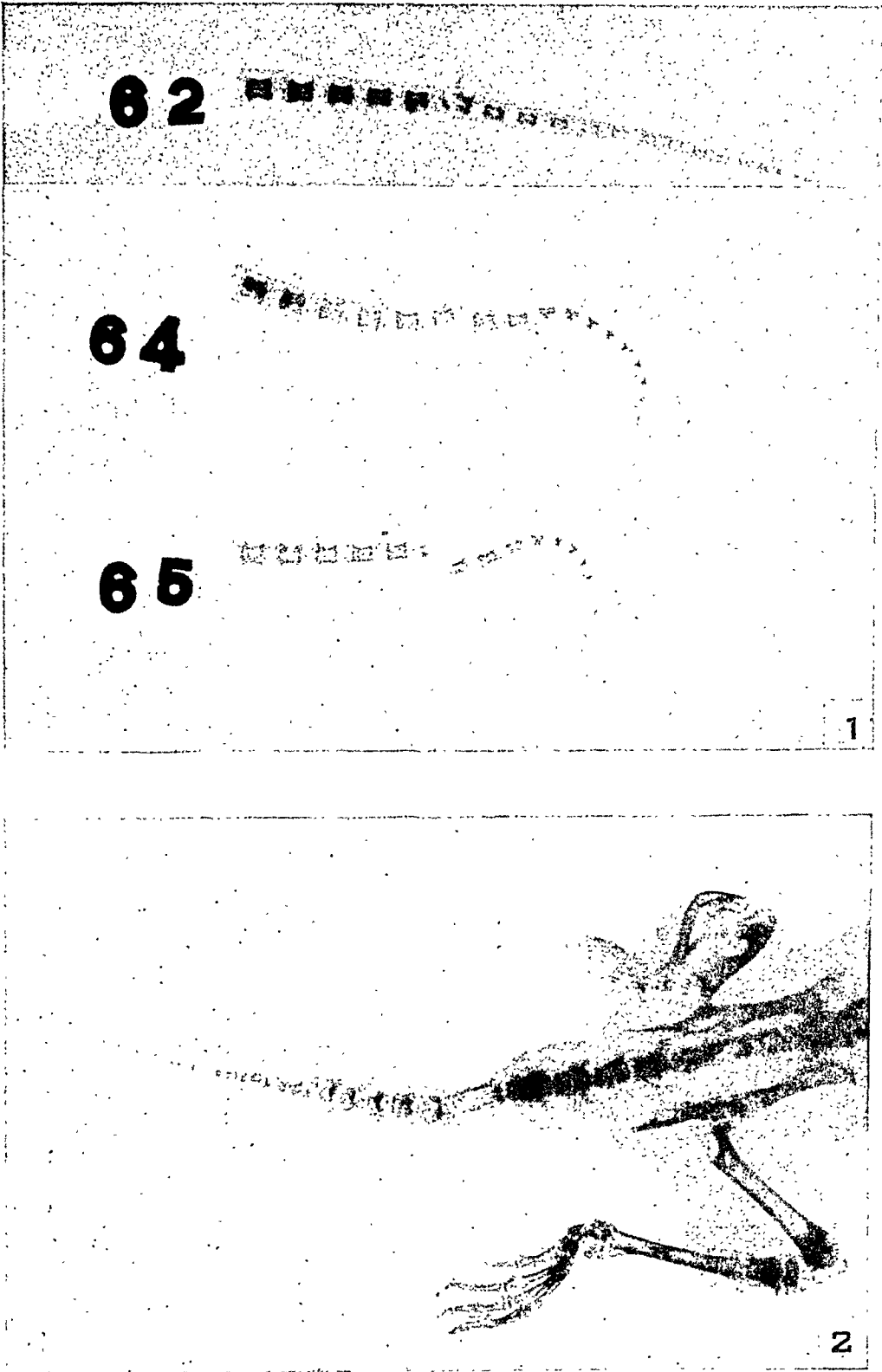
[ Illustrations follow ]

## DESCRIPTION OF PLATES

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### PLATE 19

- FIG. 1. Rat 62. Roentgenogram of the tail of a 20-day-old normal rat. Rats 64 and 65. Roentgenogram of the tails 20 days after brief exposure of the tail to cold on the first day of life. Of note are the wedge-shaped vertebrae associated with the angulation of the tail. (See Fig. 7 for histologic section.) Natural size.
- FIG. 2. Roentgenogram taken 38 days after the tail, left knee joint, and both feet were held against a cold surface for about 1 minute on the first day of life. Shortening and curvature of the shaft of the left femur and tibia adjacent to the knee joint are seen. The more distal caudal vertebrae also show evidence of damage by their shortness, irregular calcification, and asymmetry. (See Fig. 8 for histologic section of left knee joint, and text for discussion.) Natural size.



Scow

Destruction of Cartilage Cells by Refrigeration



## PLATE 20

FIG. 3. Longitudinal section of caudal vertebrae of a normal newborn rat. (There is a remnant of the notochord in the midline of the vertebrae connected to the nucleus pulposus of the intervertebral disk.) Hematoxylin and eosin stain.  $\times 10$ .

The key to the numbers on the diagrammatic tracings of Figures 3 to 7 is as follows:

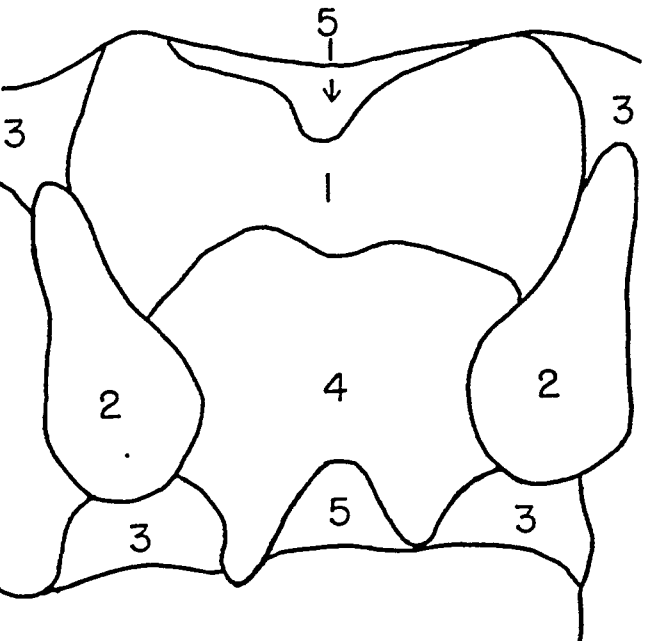
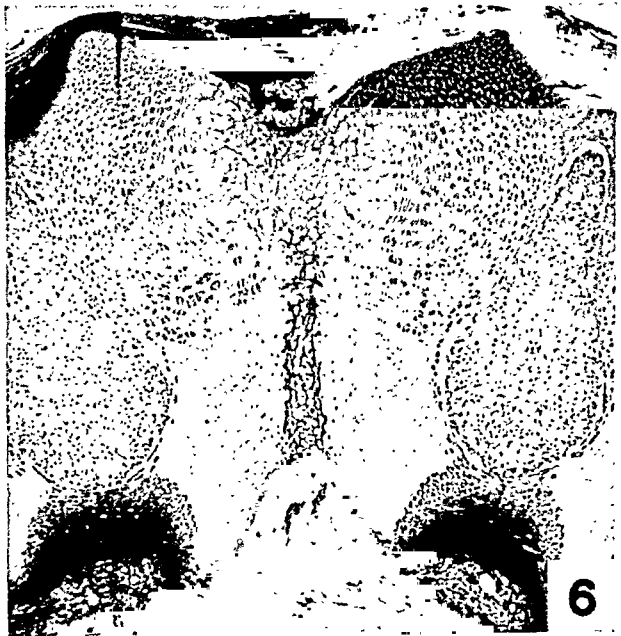
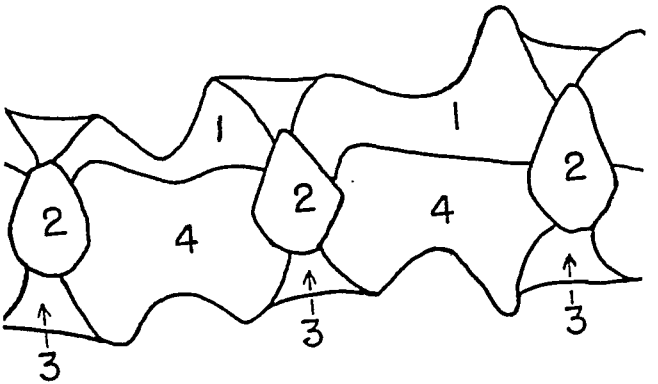
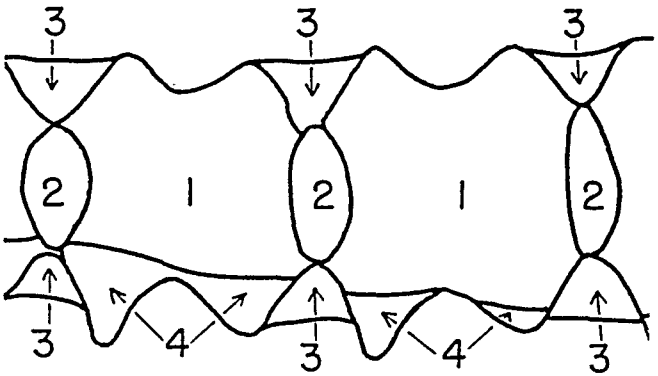
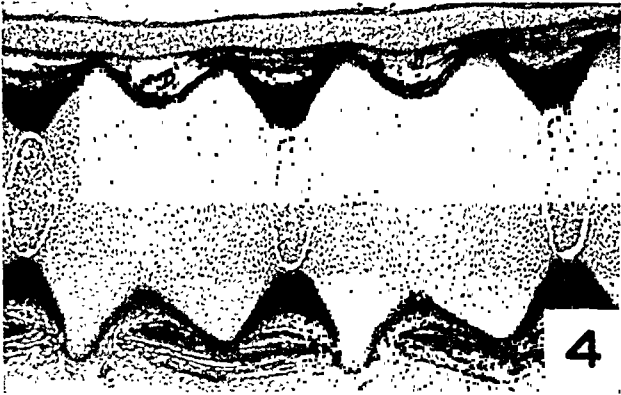
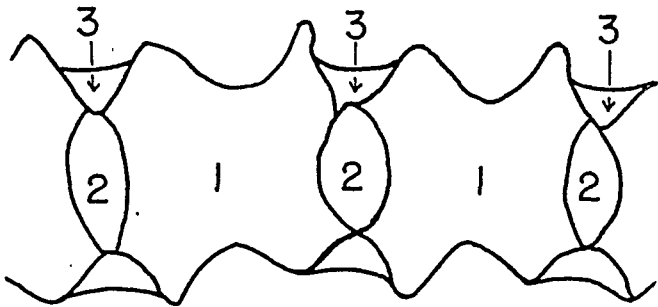
1. Normal, unossified caudal vertebra (cartilage)
2. Nucleus pulposus
3. Annulus fibrosus
4. Portion of unossified caudal vertebra (cartilage) which has been damaged by freezing
5. Bone developed by periosteal ossification
6. Cartilage taking active part in endochondral ossification
7. Bone developed by endochondral and periosteal ossification

FIG. 4. Longitudinal section of the tail vertebrae of a newborn rat sacrificed  $3\frac{1}{4}$  hour after brief exposure of the tail to cold. The necrotic cells with shrunken nuclei and cytoplasm seen in area 4 may be compared with normal cartilage cells in area 1. Hematoxylin and eosin stain.  $\times 10$ .

Tail vertebrae seen in Figures 4, 5, 6, and 7 were all similarly treated. The side of the tail seen in the lower portion of the photomicrograph was held gently for 10 seconds against an ice-cold surface (see text).

FIG. 5. Longitudinal section of caudal vertebrae of a rat sacrificed  $2\frac{1}{2}$  days after the tail was frozen on the first day of life. Hematoxylin and eosin stain.  $\times 10$ .

FIG. 6. Longitudinal section of caudal vertebrae of a rat sacrificed 4 days after brief freezing of the tail on the first day of life. Of note is the wedge-shaped vertebra with migration of nucleus pulposus (area 2) to the damaged side. Apparently normal development of vertebra may be observed on the uninjured side. Hematoxylin and eosin stain.  $\times 10$ .

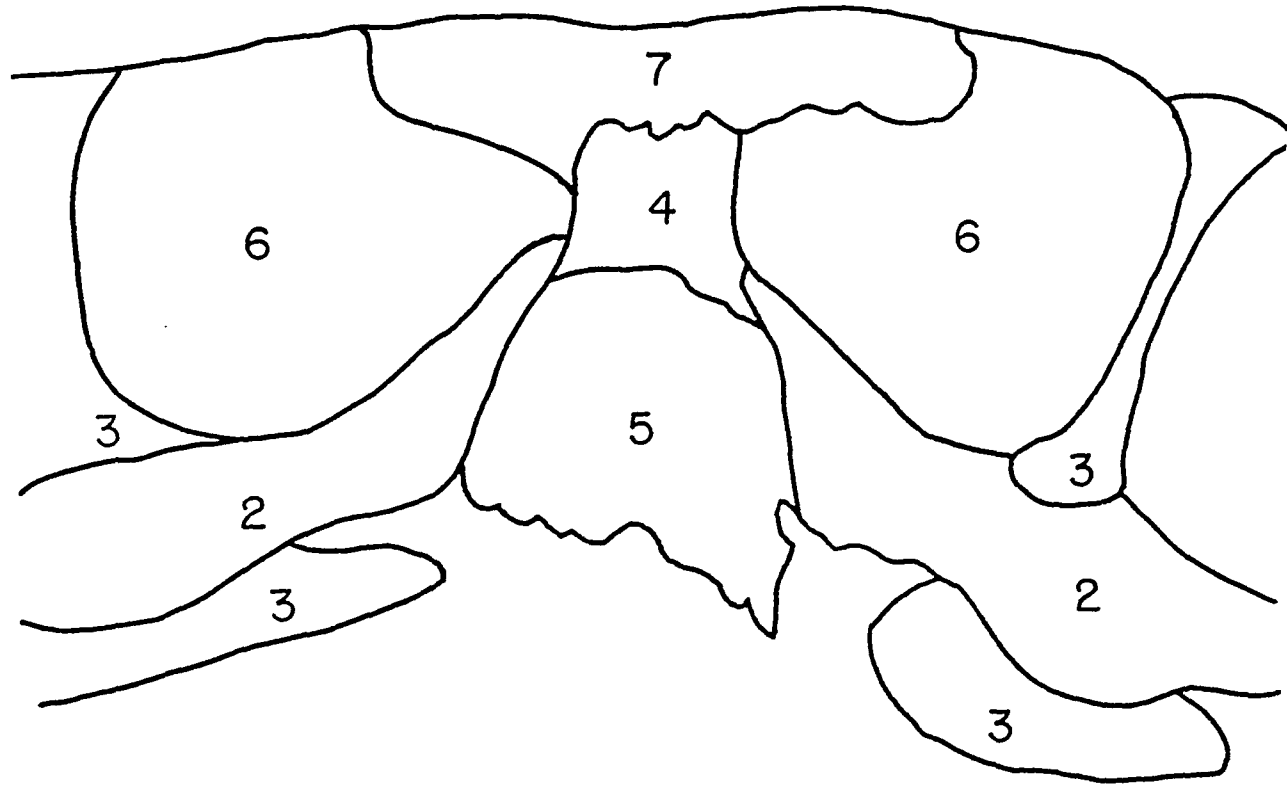


Scow

Destruction of Cartilage Cells by Refrigeration

PLATE 21

FIG. 7. Longitudinal section of a tail vertebra of a rat sacrificed 20 days after brief exposure of the tail on the first day of life. "Herniation" and migration of the nucleus pulposus (area 2) to the injured side may be observed, yet this area retains its connection with the remnant of injured cartilage (area 4). Of note is the difference between the length of the injured portion of the cartilage and that of the normally growing vertebra at the top of the photomicrograph (the uninjured side). Hematoxylin and eosin stain.  $\times 10$ .



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Destruction of Cartilage Cells by Refrigeration

PLATE 22

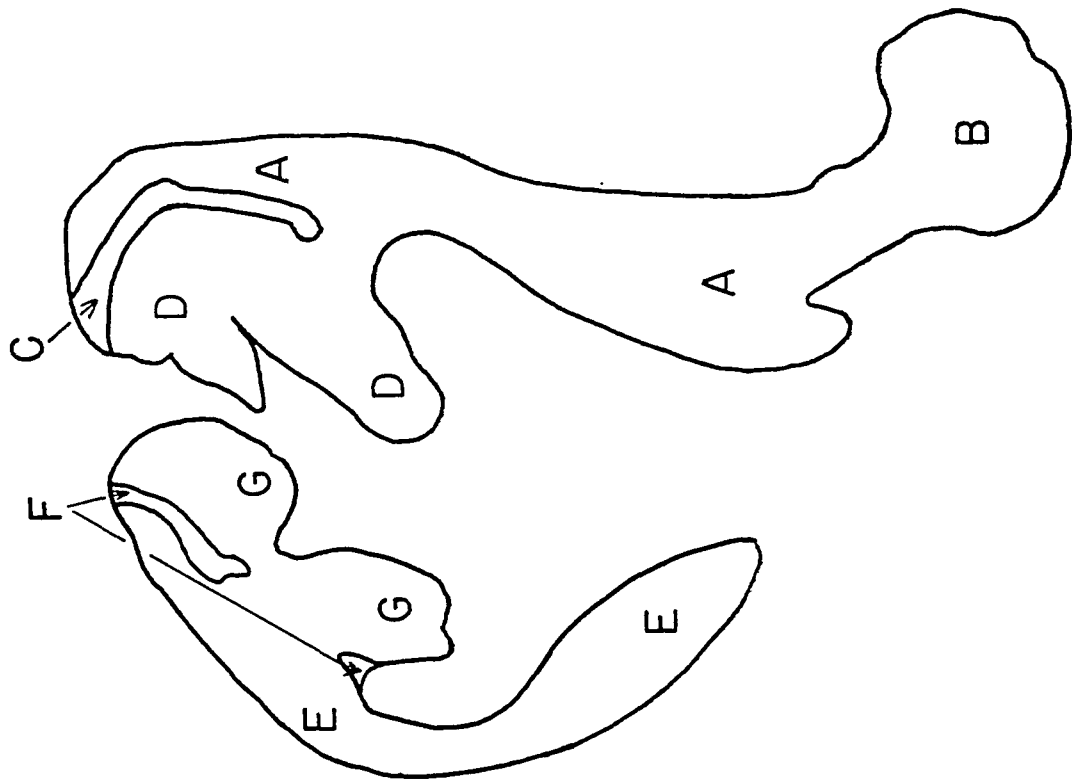
FIG. 8. Longitudinal section of the left femur and tibia of a 38-day-old rat, which were held against a cold surface for 1 minute on the first day of life. (See roentgenogram in Fig. 2.) Of note are the curvature of the shaft (A and E), interruption of epiphyseal lines (C and F) adjacent to knee joint, and rotation of articular surfaces of the two bones (see text for further description). Hematoxylin and eosin stain.  $\times 3.5$ .

The key to lettered areas is as follows: Femur: A, shaft; B, head; C, distal epiphyseal line; and D, condyles. Tibia: E, shaft; F, proximal epiphyseal line; and G, condyles.



Scow

B



Destruction of Cartilage Cells by Refrigeration



# EFFECT OF DIET DURING PREGNANCY UPON THE INCIDENCE OF CONGENITAL HEREDITARY DIAPHRAGMATIC HERNIA IN THE RAT

## FAILURE TO PRODUCE CYSTIC FIBROSIS OF THE PANCREAS BY MATERNAL VITAMIN A DEFICIENCY \*

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The initial purpose of these experiments was to attempt to produce cystic fibrosis of the pancreas in newborn rats by means of breeding on a diet containing the minimum amount of vitamin A compatible with reproduction. The hypothesis that diet during pregnancy might play a rôle in the production of this lesion was based on three considerations which appeared valid when the experiments were begun in 1939. (1). Cystic fibrosis was known to occur in several siblings but no instance was known of its occurrence in more distant relatives.<sup>1</sup> Several such cases have since been observed by myself and others.<sup>2</sup> A disease of this pattern of incidence might be due to some untoward circumstance of pregnancy and is not necessarily of genetic origin. (2). The lesions found consist of obstruction of various epithelium-lined ducts, especially those of the pancreas. The obstruction is now believed to be the result of abnormalities in the material secreted.<sup>3,4</sup> (3). Obstruction of ducts by metaplastic epithelium had been described in experimental vitamin A deficiency.<sup>5</sup>

It seemed within the range of possibility and compatible with the facts then known, that the disease might be the result of vitamin A deficiency in the fetus. Since clinical vitamin A deficiency is not a characteristic of the mothers of patients with cystic fibrosis of the pancreas, a mechanism for the production of fetal deficiency without maternal deficiency must be postulated if this be the cause of the disease. Three explanations were devised: First, that an anomaly of maternal metabolism might lead to fetal deficiency; second, that the fetus, owing to a genetic abnormality, had an unusually high requirement of the vitamin; and third, that a degree of vitamin deficiency might exist which was adequate for the mother but inadequate for the fetus. Hence, either in the presence of a genetic metabolic anomaly or in normal animals, there might exist a level of vitamin A in the maternal diet which was compatible with maternal health and fetal deficiency.

The first step in testing this rather elaborate scheme was to demonstrate by animal experimentation that vitamin A deficiency during

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fetal life leads to cystic fibrosis of the pancreas. The following experiments failed entirely in this objective. They have, however, provided convincing support of the more general hypothesis that a level of nutritional deficiency during pregnancy may occur in which the mothers show few or no signs of deficiency but the young die in the first day or two of life. As the experiment progressed it also became apparent that another important concept had been proved, namely, that the frequency of expression of a hereditary congenital malformation may be increased by deficiency in the maternal diet.

The literature on the production of congenital malformations by means of deficient diets during pregnancy has been reviewed recently by Warkany.<sup>6</sup> The malformations which have been described as the result of breeding on diets deficient in vitamin A include a variety of anomalies of the eye,<sup>7-9</sup> the teeth,<sup>10</sup> and malformations resulting from disproportionate growth of the skeleton and nervous system, including hydrocephalus<sup>11</sup> and blindness due to constriction of the optic nerve.<sup>12</sup> A preliminary report of the occurrence of diaphragmatic hernia in the young of vitamin A-deficient rats was made by me in 1941.<sup>13</sup> No data have been found relating to the effect of deficiency of specific nutritional substances during pregnancy on the incidence of malformations in the human being. Nonspecific nutritional deficiency leads to lowered fertility and increased infant mortality.<sup>14-16</sup>

## METHODS

*Experimental Animals.* The rats used in all but one experiment were of a colony which originated in 6 white rats which were purchased in 1929 and bred in the laboratory since that time without the addition of a new strain. Breeding has not been consistently by brother and sister matings. The rats used as breeders for the continuation of the colony have been selected on the basis of size, general condition, and freedom from infection of the first litters. Post-mortem examination of the lungs of all animals for evidence of infection has therefore been part of the routine of all experiments for the 8 years and over 3000 animals preceding the present study. This procedure has decreased the incidence of lung infections from about 50 per cent to something less than 10 per cent of the adult animals of the stock colony and has inadvertently provided evidence that diaphragmatic hernia of major degree was at least not common in the strain under routine breeding conditions. No instances of this anomaly were observed and if they had been common they could hardly have been overlooked.

The rats used in one experiment (no. VIII) were of the Long-Evans

strain. They were bred from 2 females and a male obtained in 1939. Breeding was not exclusively by brother-sister matings but care was taken to breed only within the strain. By chance the original rats were all black, although some of the young were hooded. Black rats were selected as breeders, so that the rats used in experiment VIII were black. This precaution was taken to minimize the chance of admixture with the white stock colony.

In order to ascertain fertility and also the incidence of resorption of the fetuses and the duration of pregnancy, all matings were carried out after a few days or weeks of examination of the vaginal smear. The rats were mated only when the smear was of the estrous type and they were examined on the following day for sperm or plug. After positive mating the male was removed from the cage and the female was weighed. She was weighed subsequently about twice a week. Infertility was nearly always the result of failure to have normal estrous cycles, the more deficient animals continuously showing cornified epithelium in the smears. Occasional resorptions were observed. The chief complication of pregnancy was delayed parturition, which has previously been reported in vitamin A-deficient rats.<sup>17</sup>

*Diets.* The stock diet used throughout was as follows:

Whole wheat flour .....	670 gm.
Casein .....	150 gm.
Dried milk .....	100 gm.
Calcium carbonate .....	15 gm.
Sodium chloride .....	10 gm.
Butter .....	50 gm.
Brewer's yeast .....	5 gm.
<hr/>	
1000 gm.	

In this diet the main source of vitamin A was butter and the amount of the vitamin was somewhat variable. The batches of diet were made up weekly and kept in closed tins. This diet has been used routinely for the stock colony.

The vitamin A-deficient diet, diet 30, was as follows:

Casein, defatted .....	180 gm.
Cornstarch .....	570 gm.
Vegetable fat (crisco) .....	50 gm.
Wheat germ .....	50 gm.
Salt mixture .....	50 gm.
Brewer's yeast .....	100 gm.
Viosterol .....	15 drops
<hr/>	
1000 gm.	

The casein was subjected to continuous extraction with hot 95 per cent ethyl alcohol for a total of 20 hours over a period of 3 days by the method described by Sperry.<sup>16</sup> After 3 to 6 hours of extraction the casein was removed from the extraction bag, ground, sieved, and replaced. After conclusion of the extraction it was dried in air, sieved, and stored in cans until use. In the preparation of the diet the dry ingredients were first mixed. The viosterol was worked thoroughly into the crisco and this was then worked into the dry materials. The diet was made up weekly. It was not assayed for vitamin content but this was apparently adequate except for vitamin A, since rats showed normal growth and reproduction for many generations when given diet 30 supplemented by vitamin A.

Haliver oil (Abbott) was used as the source of vitamin A. Various dilutions were made with Wesson oil, the calculations being based on the manufacturer's assay (1 gm. = 50,000 international units; 1 gm. = 40 drops; 1 drop = 1250 i.u.) The various dilutions used were given letters for convenience, with the following estimated content of vitamin A: X, 625 i.u. per drop; Z, 125; A, 25; B, 5; C, 1; D, 0.2; E, Wesson oil.

The supplement was given in amounts of 1 drop per day per rat and was fed three times a week by medicine dropper. Care was taken that only rats receiving the same supplement were kept in the same cage, but no attempt was made to prevent coprophagy.

#### PRELIMINARY EXPERIMENTS TO DETERMINE THE MAXIMUM DEFICIENCY OF VITAMIN A COMPATIBLE WITH REPRODUCTION

A series of experiments was carried out with small numbers of animals in order to determine the lowest dietary level of vitamin A compatible with fertility and the birth of young. In these experiments the factors to be considered were the age at which the rats were transferred from the stock diet to diet 30, the age at mating, the daily supplement of vitamin A, the length of time during which it was given, and the relation of this period to pregnancy and parturition.

*Experiment I.* At the age of 70 days, 12 female rats were transferred from the stock diet to diet 30. At 90 days they were divided into five groups and these were given supplements A, B, C, D, and E respectively. The rats were mated and all bore litters at ages between 113 and 142 days. Three litters were missing on the day after birth (on B, C, and D levels). The rest showed no abnormalities except for a lower average weight at weaning in the rats bred on the lower supplements. It was concluded that the rats were started on the diet too late to be adequately depleted of vitamin A.

*Experiment II.* Nineteen female rats were started on diet 30 at the age of 35 days and the supplements Z, A, B, C, and E were started at the age of 60 days, each level of supplement being given to 3 of 4 rats. Mating was begun at 80 days. Estrous cycles were irregular and fertility

was subnormal. However, 11 of the 12 rats receiving Z, A, or B levels became pregnant. None of the rats receiving C or E supplements were successfully mated. After positive mating the supplement was discontinued.

The 54 young of the 7 rats receiving the supplements A or Z appeared normal on the day of birth but disappeared one at a time during the first week, so that only 10 survived to the age of weaning (21 days). No external abnormalities were noted, and few of the young were autopsied since only fragments of them were found. Of the 4 rats on the B supplement, 2 died during parturition and the other 2 gave birth. One litter was born during the night and had been eaten by morning. The other litter was dead when found; *3 of its 7 members were found to have hernias of the right side of the diaphragm* and no abnormalities were found in the other 4.

From this experiment the conclusion was drawn that the initial depletion of vitamin stores was adequate and that the supplements at the Z, A, and B levels were sufficient to permit positive mating but that the total deficiency at the end of pregnancy in rats receiving B supplement was too great for survival of the young, and insufficient vitamin was stored at the A and Z levels of intake to provide for the demands of pregnancy and lactation when the vitamin was withheld after positive mating. The occurrence of one litter with 3 rats having diaphragmatic hernia was considered a chance finding.

*Experiment III.* It was apparent that the deficiency was not great enough for the production of abnormal young in experiment I and too great for normal parturition and survival of the young in experiment II. Experiment II was repeated, using the surviving young of the rats of Experiment I, to evaluate the rôle of the diet of the previous generation. The results were essentially similar to those of experiment II. The chief contribution of this experiment was the finding of *right diaphragmatic hernia in 3 of the 17 surviving young rats*.

*Experiment IV.* Experiment IV was designed to discover whether the neonatal death of the young in the previous experiments was due to inadequate lactation and also whether supplement given late in pregnancy would increase the proportion of young surviving. The breeding mothers were composed of three groups: *a.* One group was placed on diet 30 at 35 days and mating was begun at 70 days; supplement B was begun on the 60th day and continued through pregnancy. *b.* A second group was placed on diet 30 at 30 days and given no supplement until the 16th to 21st day after successful mating when they received an initial dose of 4 drops of "Z" followed by 2 drops on alter-

nate days. *c.* A third group used as controls was kept and bred on the stock diet. The plan was to breed one of the control rats on the day following each positive mating of one of the deficient animals, and exchange half of each litter at birth or the following day. The control young were marked by clipping the end of the fifth toe of the right fore-

TABLE I

*Experiment IV. The Results of Exchanging Half of Each Litter of the Vitamin A-Deficient Rats with Half of Each Litter of Controls, to Determine the Role of Lactation in the Neonatal Mortality.*

Experiment	Nursed by rat no.	Young of rat no.	No. of young	Mean wt., gm., at		
				1-3 days	14 days	21 days
IV-a	3618 Deficient	3618 3601	3 (1 dead at 8 days) 4	6 (2 days) 7.25	17.5 18.25	24.5 21.75
	3601 Control	3618 3601	2 5 (1 dead at 8 days)	6.0 8.8	26.5 27.75	37.5 39.5
IV-a	3639 Deficient	3639 3644	4 4	5.25 (1 day) 5.25	Dead by	
	3644 Control	3639 3644	4 4	5.25 5.25	4th day 26.25	44
IV-b	3669 Deficient	3669 3660	3 (1 dead at 2 days) 4	6.0 (3 days) 7.5	17.0 18.75	32.0 36.0
	3660 Control	3669 3660	3 4	7.3 9.0	25.0 27.0	44.3 49.0
IV-b	3696 Deficient	3696 3661	1 4	Dead (2 days) 10	28.5	49
	3661 Control	3696 3661	3 4	Dead (2 days) 10		45

foot. Each control mother therefore suckled half the litter of the experimental animal and half of her own litter, and each experimental mother likewise had a mixed litter. When necessary, young rats were discarded so as to have no more than 8 in each litter.

This plan contained too many hazards and was successfully executed in only 2 cases of the *a* and 2 of the *b* groups, a total of 4 successes in 14 rats started on the experiment (Table I). Of the remainder, one proved sterile, 2 resorbed their litters, success in obtaining parallel control litters was lacking in 2, and the remaining 5 litters were born dead.

In the four experiments in which the exchange of litters was safely made, the results were varied. In the first, both litters were weaned, with obviously better growth in the litter cared for by the control mother. In the second, the entire mixed litter cared for by the experi-

mental animal and also her young cared for by the control mother died within a few days. The results in the third pair of rats paralleled those of the first, and in the fourth all of the offspring of the experimental mother died by the second day while all those of the control mother did well, whichever mother cared for them. Thus an example of each possible result was observed. The answer to the question whether

TABLE II

*Experiment IV. Incidence of Diaphragmatic Hernia in the Young of Rats Bred on Minimal Amounts of Vitamin A Compatible with Reproduction. The Rats Were Placed on Diet 30 at 35 Days of Age. Group a Received Supplement B from the 60th Day, Group b Received Supplement Z Beginning at the 16-19 Day of Pregnancy.*

	Rat	Z given on day	Number of offspring			
			Born	Survived to 21st day	Autopsied	With D.H.
Group a	3618	—	7	4	7	1
	3639	—	8	0	1	0
	3641	—	4	0	3	1
	3621	—	7	0	7	2
	3626	—	6	0 (4 killed)	4	1
	3645	—	8	6	6	0
	3628	(resorption)				
Total			40	10	28	5
Group b	3669	16	7	6	5	0
	3696	18	7	0	3	1
	3654	19	5	0	5	5
	3658	19	9	0	9	9
	3694	18	12	0	12	0
	3664	Resorption				
	3665	No successful mating				
Total			40	6	34	15
Grand Total			80	16	62	20

neonatal death was due to deficient lactation was not decisive, but observation of the young led to the belief that the majority of the young of the deficient mothers were too feeble to suckle.

Although the objective of this experiment was not attained, in the course of the careful watch necessary to carry out the experiment, for the first time a large percentage of the young were rescued from maternal ingestion. In the course of post-mortem examination of the young of the experimental animals an important observation was made. *Hernia of the diaphragm was found in a total of 20 of the 61 animals examined, most of them from litters of the b group in which supplement was withheld until late in pregnancy (Table II). All of the young of 2 litters, numbering 5 and 9 respectively, showed a hernia of the diaphragm of severe degree.*

## EXPERIMENTS DIRECTED AT DIAPHRAGMATIC HERNIA

*Experiment V.* The first move in experiment V was to attempt to reproduce the lesions observed in experiment IV-*b*. This and subsequent experiments were based on the following standard procedure. The rats were taken from the stock colony and placed on diet 30 at the age of 35 to 42 days. Beginning at about the 70th day a vaginal smear was examined each day. When the smear was of the estrous type the rat was left overnight with a normal male which had been bred on an adequate diet. On the following morning a vaginal smear was examined for sperm or a plug. The male was then removed. The female was weighed on this day and twice a week thereafter. On the 16th to 18th day following the day on which the male was placed in the cage, the female was weighed and if there had been enough gain in weight to indicate pregnancy, the rat was given 4 or more drops of Z supplement by medicine dropper (500 i.u. of vitamin A) and 2 drops every 2 days thereafter. When mating was unsuccessful and vaginal smears showed a continuous production of cornified epithelium without estrus, 2 drops of Z supplement sometimes were given, and this often sufficed to produce an estrous cycle and positive mating.

In experiment V, 14 rats were thus treated, together with 14 controls which also were placed on diet 30 but received Z supplement regularly from the 60th day of age and an additional 12 drops late in pregnancy between the 17th and 20th day. Four of the deficient rats failed to become pregnant and were found to have pulmonary infection. One positive mating was followed by a resorption. Nine litters were born, totaling 69 rats. Two entire litters and members of 5 others were found dead on the day of birth, a total of 22 rats. A careful watch was kept so that all rats except 2 were recovered before maternal ingestion. All living young were killed at once and all 67 of the recovered rats were autopsied (Table V). Twelve rats of 4 litters showed a diaphragmatic hernia. Eleven of the control rats gave birth to litters, totaling 78 young; of these, 4 rats, one from each of 4 litters, had hernias. The malformation could therefore be reproduced in a fair proportion of the young bred on the standard regime, but it occurred also in the control litters.

*Experiment VI.* In experiment VI further proof that a deficiency of vitamin A increased the incidence of the malformation was sought by several methods. The first of these was the breeding of 5 successive generations of rats on diet 30 with supplement Z, to determine the incidence of hernia and also to test severely the adequacy of the diet. The sixth generation was divided into 2 groups, one of which was placed

on diet 30 + E and the other, composed of littermate controls, on diet 30 + Z, the supplements being started at 60 days of age. Facilities did not permit the maintenance and breeding of all rats of 6 generations. F<sub>1</sub> consisted of 8 females and 4 males picked at random from the stock colony; therefore the experiment was not based strictly on inbreeding. In subsequent generations, brother and sister matings were frequently but not uniformly made; breeding was always with the animals of the

TABLE III

*Experiment VI. The Incidence of Diaphragmatic Hernia in the Young of Rats Bred for Five Generations on Diet 30 + Z as Compared with the Sixth Generation, Half of Which Were Bred on Diet 30 with Supplement and Half Without. All but One of the F<sub>6</sub> Experimental Rats Had Ceased to Have Estrous Cycles and a Single Estrus Was Induced by One or More Doses of Two Drops of Z (250 i.u. of Vitamin A).*

Generation	Bred on diet	Number of litters		Number of rats	
		Total	With D.H.	Total	With D.H.
F <sub>0</sub>	Stock			8	1
F <sub>1</sub>	30 + Z	8	2	35	2
F <sub>2</sub>	30 + Z	7	1	34	2
F <sub>3</sub>	30 + Z	9	2	60	2
F <sub>4</sub>	30 + Z	9	3	51	6
F <sub>5</sub>	30 + Z	9	1	35	2
F <sub>6</sub> C	30 + Z	14	1	108	1
Total	Diet 30 + Z	56	10	323	15
F <sub>6</sub> Exp.	Diet 30	14	5	92	17

same generation for this experiment. In the first 5 generations a number of young were saved for breeding and the remainder killed. All were ultimately autopsied. Of a total of 415 rats, 14 were found to have a diaphragmatic hernia (Table III). In the sixth generation the females of 8 litters were distributed into 2 groups of 15 rats each, dividing the littermates as evenly as possible. There were 12 pairs of littermates. Fourteen rats of each group produced litters. The young were killed on the day of birth to prevent destruction of the evidence. Of the 92 rats in the experimental litters, 17 had hernias of the diaphragm, while one of the 108 rats in the control litters had this malformation (Table III). Therefore, it was apparent that, although the appearance of hernia was suppressed in large part for successive generations on an adequate diet, it reappeared in a generation given a deficient diet. The adequacy of diet 30 + Z supplement is attested by the high fertility and good growth and appearance of the rats maintained on it for 6 generations.

*Experiment VII.* In experiment VII, wherever possible, the rats



which had produced a number of young with hernias when bred on a deficient diet were given supplement Z three times a week at levels of 7 drops weekly for several weeks, while diet 30 was continued. They were then bred again, if possible to the same males, and the litters examined for hernias. In no instance did these second litters include a rat with a hernia (Table IV). The tendency to herniation was apparently suppressed in the presence of high dietary levels of vitamin A.

TABLE IV

*Experiment VII. Comparison of the Incidence of Diaphragmatic Hernia in the Litters of Rats Maintained on a Deficient Diet with Subsequent Litters of the Same Rats after Vitamin Supplements Had Been Given.*

First litter, diet 30; no supplement until late in pregnancy						Second litter, diet 30 with Z supplement					
Female	Male	Age	No. of young	No. for post-mortem	No. with D.H.	Z supplement from age	Male	Age	No. of young	No. for post-mortem	No. with D.H.
		days				days		days			
3654	3598	99	5	5	5	95	3598	136	2	1	0
3658	3597	99	9	9	9	94	3597	135	11	4	0
4413	4174	147	9	9	6	142	4157	188	9	9	0
4424	4174	142	6	6	1	141	4162	199	8	8	0
4429	4132	144	6	6	1	143	4162	184	9	8	0
4442	4117	104	11	11	8	100	4157	190	9	8	0
4297	3946	135	2	2	1	128	3953	238	3	3	0
4307	4112	130	7	7	3	124	4024	231	5	5	0
4333	4226	94	6	6	2	89	4226	158	9	9	0
Total 9 litters			61	61	36	9 litters			65	55	0

Comparison of the incidence of hernias among autopsied rats in first and second litters gives  $\chi^2 = 51.3$ ,  $P = < .01$ .

*Experiment VIII.* In experiment VIII, in order to determine whether the appearance of hernias under these dietary conditions was a characteristic confined to our inbred strain of stock rats, experiment VI-b was twice repeated, using inbred rats of the Long-Evans strain. These rats were black, having been selectively inbred in this regard for the purpose of making sure that there was no accidental admixture with the white stock colony. The first batch was run in April and May and the second in September and October, 1941. The data are presented in Table V. One hernia was found in the young of one of the autumn experimental group and none were found in the controls. There is thus a marked difference in these two strains of rats in the tendency for diaphragmatic hernia to appear under the influence of vitamin A deficiency.

*Experiment IX.* An attempt was made in experiment IX to discover the genetic characteristics of the malformation, by producing a sub-

strain in which the incidence of hernias was uniformly high under experimental conditions. For this purpose female rats nos. 3654 and 3658 were chosen (Table VI). These rats had been part of experiment IV-*b* and had produced 5 and 9 young, respectively, all of which had

TABLE V

*Comparison of the Incidence of Diaphragmatic Hernia in the Stock, D.H., and Long-Evans Strains on Diet 30 with and without Z Supplement.*

Strain	Experiment	Experiment or control	Rats	Litters	No. autopsied	No. with D.H.	Percentage with D.H.	X <sup>2</sup> and P
Stock	V	Experiment	14	9	67	12		
		Control	14	11	78	4		
	VI F <sub>0</sub>	Experiment	15	14	92	17		
		Control	15	14	108	1		
	Total	Experiment	29	23	159	29	18.2	X <sup>2</sup> = 23.4
		Control	29	25	186	5	2.7	P = <.01
D.H.	Carotene experiment	Experiment	9	7	34	14		
		Control	8	8	46	5		
		Experiment	12	8	55	16		
		Control	12	11	84	5		
	Total	Experiment	21	15	89	30	33.7	X <sup>2</sup> = 23.2
		Control	20	19	130	10	7.7	P = <.01
Long-Evans	Spring	Experiment	12	10	68	0		
		Control	13	13	87	0		
	Fall	Experiment	11	8	43	1		
		Control	11	11	66	0		
	Total	Experiment	23	18	111	1	0.9	
		Control	24	24	153	0	0	P = <.01

Comparison of the stock and D.H. strains on the experimental regime gives  $X^2 = 7.53$ ,  $P = <.01$ ; and of these strains on the control diet comparison gives  $X^2 = 5.5$ ,  $P = <.02$ . The infrequency of hernia in the Long-Evans strain is apparent.

had right diaphragmatic hernias, 13 of them complete. These rats had been littermates from the stock colony and had been placed on diet 30 at 36 days of age. They were mated to males nos. 3598 and 3597, respectively, which were littermates. All 4 of these rats were without hernias. After their participation in experiment IV-*b*, the females were continued on diet 30 and given 7 drops of Z weekly and partook in experiment VII, producing small litters in which no hernias were found. They were then both placed on stock diet and after several weeks were each bred twice more, all 4 litters being sired by rat no. 3598. These 3 rats, 3654, 3658, and 3598, are considered as the F<sub>1</sub> generation of the D.H. strain. The resulting strain produced a larger number of members with hernias than the original stock strain, but the incidence still varied with the diet. With the use of this D.H. strain some infor-

mation as to genetic pattern of the malformation was obtained (Text-Fig. 1 and Table VII). On the stock diet in a total of 328 rats, 107 (or 32.6 per cent) were found to have a hernia. The sex distribution was approximately equal, with 50 of 164 males and 57 of 156 females affected; the sex was not recorded in 8 unaffected rats. Of these hernias,

TABLE VI

*Breeding Record of Rats 3654 and 3658, of Experiment IV. The D.H. Strain Was Derived from Litters 3 and 4.*

Female	Litter	Male	Diet	Supplement	Parturition		No. born	No. for post-mortem	No. with D.H.
					Age	Day of pregnancy			
3654	1	3598	30	4 Z at 19th day	days 99	24	5	5	5
	2	3598	30	7 Z per week	136	24	2	1	0
	3	3598	Stock	0	217	24	9	7	1
	4	3598	Stock	0	308	?	4	4	1
3658	1	3597	30	2 Z on 19th day	99	25	9	9	9
	2	3597	30	7 Z per week	135	23	11	4	0
	3	3598	Stock	0	219	23	10	10	3
	4	3598	Stock	0	295	23	9	9	4

34 were pericardial, 13 were right incomplete, and 3 were right complete in the males; 49 were pericardial, 6 were right incomplete, and 2 were right complete in the females, a sex difference which is probably not significant.

The incidence of hernias in the young is significantly greater when both parents are affected than when neither is affected, while the inci-

TABLE VII

*Experiment IX. Incidence of Hernias in the Young of the D.H. Strain in Relation to the Presence of Hernia in the Parents. Stock Diet.*

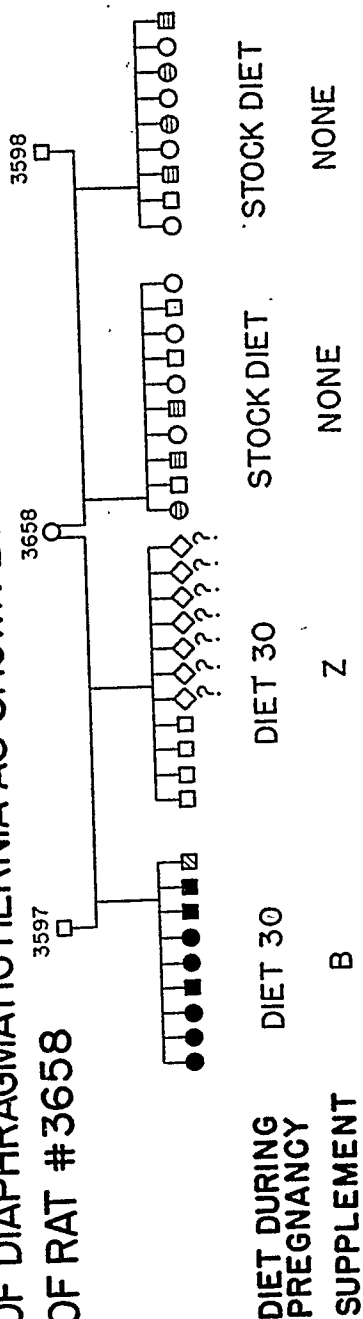
Hernia in parents		No. of litters	Young						Total		Percentage with D.H.	
			Normal		Peric. D.H.		Other D.H.					
m.	f.		m.	?	f.	m.	f.	m.	f.	Rats		D.H.
None	None	19	50	4	37	7	12	3	3	116	25	21.6
Peric.	None	14	16	4	19	8	9	1	5	85	27	31.8
None	Peric.	4	10	9	3	1	0	0	0			
Peric.	Peric.	16	27	25	8	17	8	0	85	33	38.8	
?	None	5	11	9	8	10	4	0	42	22	52.4	
Total		58	114	8	99	34	49	16	8	328	107	32.6

Comparison of incidence of hernia in young when both parents are affected in contrast to neither parent affected gives  $X^2 = 7.1$ ,  $P = < .01$ . Comparison of litters of one versus no affected parent gives  $X^2 = 2.6$ ,  $P = < .10$ . The former is significant, the latter is not.

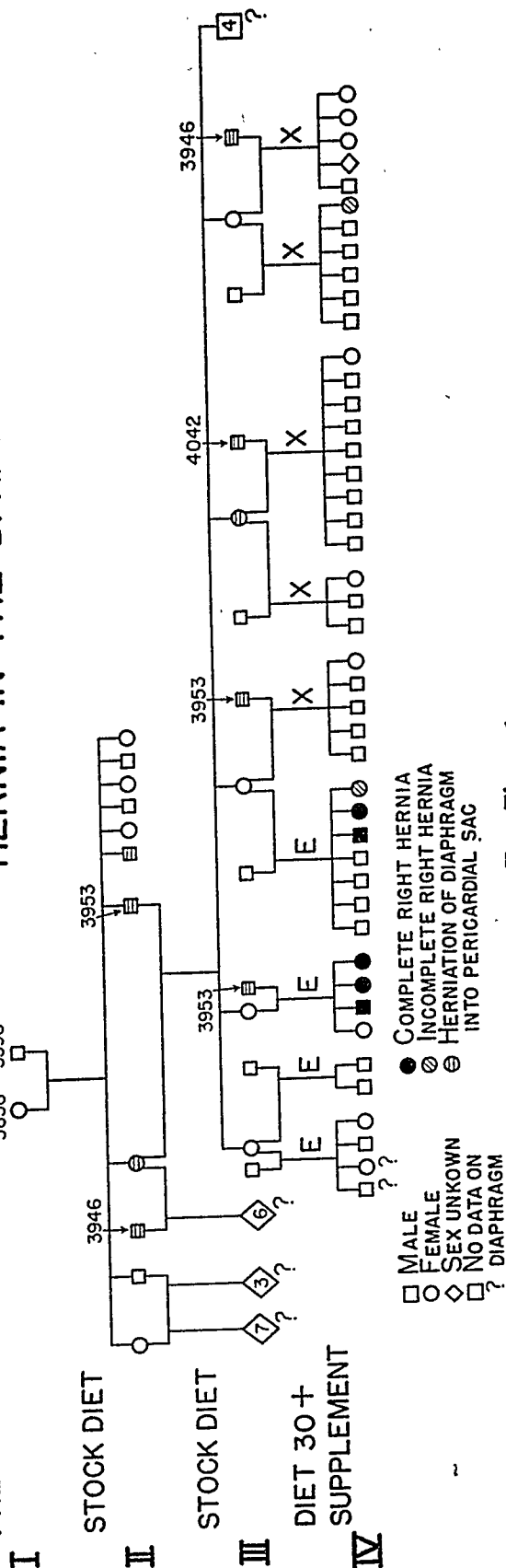
Peric. = pericardial hernia.

dence when only one parent is affected is intermediate between these two. The genetic pattern is therefore neither recessive nor dominant

# THE EFFECT OF DIET DURING PREGNANCY ON THE INCIDENCE OF DIAPHRAGMATIC HERNIA AS SHOWN BY FOUR SUCCESSIVE LITTERS OF RAT #3658



## PEDIGREE OF THIRD LITTER OF RAT #3658 ILLUSTRATING EFFECT OF DIET DURING PREGNANCY ON THE INCIDENCE OF DIAPHRAGMATIC HERNIA IN THE D. H. STRAIN



Text-Figure 1.

and is not that of a sex-linked character. The malformation appears to be either the result of a general tendency within the strain or dependent on a number of genes.

*Experiment X.* The dietary factor which suppresses the expression of the hernia has been assumed to be lack of vitamin A, because no other nutritional substance is known to be present in effective quantities in the small amounts of haliver oil which were used. At the time that these experiments were performed, crystalline vitamin A was not available. Beta carotene therefore was used as a source of vitamin A

TABLE VIII

*Experiment X. The Incidence of Diaphragmatic Hernia in the Young of Rats of the D.H. Strain Given Diet 30 with Supplements E, Z, or Carotene.*

Supplement	No. of litters	Rats			Type of D.H.			
		No. D.H.	With D.H.	Percentage with D.H.	Peric.	Rt. compl.	Rt. inc.	Left inc.
Carotene	10	63	11	17.5	0	2	8	1
30 + Z	11	79	6	7.6	1	0	5	
30 + E	8	39	16	41.0	0	4	12	

Comparison of the Groups

Supplement	X <sup>2</sup>	P
E vs. Z	12.4	<.01
E vs. B carotene	3.9	<.05
Z vs. B carotene	2.5	>.10

in an experiment designed to settle this point (experiment X). Rats of the D.H. strain were used, and were divided into 3 groups. They were subjected to the standard experimental procedure, starting the diet and supplements on the 30th day and mating on the 80th day. The supplements used were E, Z, and beta carotene dissolved in Wesson oil. The carotene solution contained 3 mg. of carotene per cc., and on the basis of 0.6  $\gamma$  of carotene being taken as the equivalent of 1 i.u. of vitamin A, this solution had a theoretical potency equal to that of the Z supplement. In the ensuing experiment, hernias were found in 41 per cent of the young of the rats receiving E, in 7.6 per cent of those receiving Z, and in 17.4 per cent of those receiving carotene (Table VIII). The difference between the incidence of hernias in the Z and E groups is statistically significant but the difference between the carotene and E groups is less striking.

As the experiment progressed it became apparent that carotene was not as effective as Z in suppressing the appearance of hernias, and it seemed possible that the difference lay in the utilization of the two substances. The livers of the remaining rats were assayed for their content of carotene and vitamin A; although the data are few, they sup-

port the hypothesis that carotene was poorly utilized (Table IX). The results of this experiment support, but do not prove, the hypothesis that the effective agent is vitamin A. It is to be regretted that it was not possible to repeat this experiment with crystalline vitamin A.

TABLE IX

*Experiment X. Assays of the Liver for Carotene and Vitamin A. Diet 30 with Supplements E, Z, or Carotene.*

Rat no.	Hernia		Supplement	Liver					Young
	m.	f.		Weight	Carotene	Vitamin A	Carotene	Vitamin A	
				gm.	$\gamma$ per gm.	i.u. per gm.	$\gamma$ per liver	i.u. per liver	
4842	None	None	E	9.8	0.45	9.3	4.4	91	Missing
4906	Peric.	Peric.	E	9.1	0.27	25.8	2.5	234	Missing
4913	?	Peric.	E	6.6	0.40	3.2	2.6	21	No litter
4835	Peric.	None	Carot.	8.6	0.95	26.1	8.1	228	7 normal, 1 rt. compl.
4836	?	None	Carot.	7.5	1.19	22.3	8.9	167	1 normal, 3?
4843	Peric.	Peric.	Carot.	6.8	0.36	37.6	2.6	264	5 normal, 1 left inc.
4881	None	None	Carot.	9.8	0.42	16.1	4.1	158	9 normal
4891	None	None	Carot.	6.0	0.55	25.4	3.3	152	5 normal, 1 rt. compl., 2 rt. inc.
4897	Peric.	Peric.	Carot.	9.3	0.33	21.8	3.0	202	Missing
4903	None	Peric.	Carot.	7.7	0.41	27.4	3.2	211	Missing
4927	None	None	Carot.	9.9	0.39	19.4	3.8	192	12 normal, 2 rt. inc.
4839	Compl.	None	Z	7.8	0.80	1767	5.9	13,783	3 normal
4847	Peric.	Peric.	Z	7.8	0.29	1463	2.2	11,411	8 normal, 1 rt. inc.
4879	?	Peric.	Z	7.1	0.60	1050	4.3	7,459	9 normal
4880	None	None	Z	10.1	0.41	853	4.1	8,614	5 normal, 1 peric., 3 rt. inc.
4892	Peric.	None	Z	7.6	0.50	987	3.8	7,498	6 normal
4904	Peric.	Peric.	Z	8.0	0.54	915	4.3	7,316	4 normal, 1 rt. inc.
4909	None	Peric.	Z	7.6	0.47	1189	3.5	9,033	10 normal
4921	None	None	Z	6.6	0.50	919	3.3	6,062	Missing

## PATHOLOGIC ANATOMY

*The Diaphragm.* The hernias of the diaphragm were classified with respect to location and type as follows: (a) Right complete, with communication between the right pleural and peritoneal cavities; (b) right incomplete, consisting of a bulge of intact diaphragm into the right pleural cavity; (c) pericardial, consisting of a bulge of the diaphragm into the pericardial cavity; (d) left incomplete. No herniation through any natural opening such as the esophageal hiatus was observed. A complete defect was found only in the right leaf. A more detailed description of the malformations follows.

(a) A right complete hernia consisted of a defect varying from one involving the posterior half of the diaphragm to one in which the right leaf of the diaphragm was lacking (Fig. 1). In some instances a narrow shelf of muscle along the anterior diaphragmatic attachment remained. In all cases part or all of the right lobe of the liver lay in the right pleural cavity, and behind it, also in the pleural cavity, there were varying amounts of small intestine and the hepatic flexure of the colon. The right lung was small and was pushed up to occupy the upper part of the cavity. The right kidney usually was in its normal position. In one instance the right horn of a pregnant uterus was incarcerated in the right pleural cavity and was the cause of death. In another instance the mass of intestines in the right pleural cavity bulged through the mediastinum behind the pericardium but anterior to the esophagus to lie partly to the left of the spine, separated by a thin membrane from the left pleural cavity.

(b) The right incomplete hernia was found always in the posterior half of the diaphragm, and consisted of a roughly hemispheric sac consisting of pleura and peritoneum without interposed muscle and containing a nubbin of liver tissue which had grown to fill it (Fig. 2). The upper surface sometimes was flattened in the larger hernias. The size varied from one measuring 10 by 10 by 5 mm., involving about one-third of the right leaf, to small protuberances about 1 mm. in diameter. The majority were between 3 and 6 mm. in diameter. Occasionally several of these small bulges were present. The location varied but was always in the posterior half of the right leaf.

(c) The pericardial hernias were similar to the smaller right incomplete hernias except that they usually were more flattened and not always round (Fig. 3). They varied from 0.5 to 7 mm. in diameter, were sometimes multiple, and always contained a nubbin of liver.

(d) The left incomplete hernias were found infrequently, lay in the posterior half of the left leaf, and usually contained the upper half of

the stomach and occasionally a loop of gut, seen through the thin membrane.

The rats having a complete hernia died in the first day or two of life with few exceptions. The incomplete hernias were commonly found in rats bred according to the standard procedure which survived to adult life, and this lesion appeared to have no effect on their health or growth. Only a small percentage of the instances of neonatal death could be attributed to a complete hernia, however, and the cause of death usually was not apparent on gross post-mortem examination.

A comparison of the frequency of hernias of the various types in the young of deficient as compared with control mothers shows a greatly increased number of right complete hernias and a somewhat greater number of right incomplete hernias in the experimental animals, but no significant difference in the pericardial and left diaphragmatic hernias. The following figures summarize the incidence of the various types in all experiments of the stock strain, but do not include the rats of the D.H. or Long-Evans strains.

<i>Type</i>	<i>Experimental rats</i>		<i>Control rats</i>	
	No.	Percentage	No.	Percentage
Right complete hernia . . . . .	32	50.8	4	16.7
Right incomplete hernia . . . . .	28	44.5	12	50.0
Pericardial hernia . . . . .	2	3.2	7	29.2
Left incomplete hernia . . . . .	1	1.6	1	4.1
Total . . . . .	63	100.0	24	100.0

Since many of these rats were observed in earlier experiments, it is probable that many of the pericardial hernias were missed, which may account for the lower incidence of this type in the stock rats as compared with the D.H. strain.

The hypothesis which most reasonably explains the morphogenesis of these hernias is a delayed growth of the diaphragm in the deficient animals. The posterior portion of the diaphragm is the last to close. Normally, the completion of the closure of the diaphragm precedes the return of the gut from the extra-embryonic celom of the umbilical cord. If the differential growth rates of the diaphragm and of the gut were altered so that the gut returned from the cord and increased in size prior to the closure of the diaphragm, it is reasonable to suppose that some abdominal organs might pass through the defect and prevent its closure.

*Pancreas and Trachea.* In the foregoing experiments it was possible to compare the microscopic changes in the trachea and in the pancreas



in rats bred on the deficient diet and examined at the age of 1 to 3 days with those in rats similarly bred but killed at weaning, in survivors continued on deficient diets, and in controls paralleling each group. It was possible to obtain sections of the organs of only a small number of rats from each experiment. These sections were stained with hematoxylin and eosin.

Of the rats bred of deficient mothers and examined at the age of 1 to 3 days, there were 8 from experiment III, 5 from experiment IV-*a*, and 8 from experiment IV-*b*, a total of 21. None showed any abnormality of the pancreas in the microscopic examination, when compared with 5 young from the stock diet controls. The trachea was examined in 4 rats of experiment IV-*a* and 7 of IV-*b*. Two of the latter showed early squamous metaplasia of the tracheal epithelium but the remaining 9 appeared normal. These 2 rats were among the 6 so examined in which a right diaphragmatic hernia was found.

Some of the rats of experiment IV in which the litters were successfully exchanged were examined microscopically, giving an opportunity to observe the effects of lactation by a deficient mother. No abnormalities of the pancreas were found in the 21 rats examined at the age of 21 to 23 days. Of these, 3 were bred and reared by deficient mothers, 4 were bred by deficient mothers and nursed by normal ones, 10 were bred by normal mothers and nursed by deficient ones, while 4 were normal controls. Squamous metaplasia of the tracheal epithelium was found in 5 of 20 rats examined, all 5 of which had been nursed by deficient mothers, but 4 were bred by normal mothers. Tracheitis was found in 9 more, 7 of which were nursed by deficient mothers. None of the rats bred and reared to weaning on a deficient diet were without either infection or metaplasia of the epithelium, while the 7 with normal tracheas were variously distributed among the other groups. Squamous metaplasia of the renal epithelium was found in only one rat, bred and reared on a deficient diet.

In addition to the above rats bred on deficient diets, there was also a large group from experiment III in which the pancreas was examined, at ages of 1 to 4 months. In none of these was there any suggestion of cystic fibrosis of the pancreas, although the acinar cells were small and atrophic in a few emaciated animals, and a few metaplastic cells were found in the pancreatic ductal epithelium in a few deficient animals. These observations were made on 12 animals bred on A supplement, 9 on B, 6 on C, 6 on D, and 8 on E supplement. In a total of 71 rats bred on levels of supplement leading to high neonatal mortality and an increased percentage of hernia (B, C, D, or E supplements), no pancreas showed changes suggestive of fibrocystic disease.

*Metastatic Calcification.* A large number of rats which had received diet 30 for a period of 10 weeks or more showed metastatic calcification. Of 44 rats which were killed before the age of 90 days and which had received diet 30 with various supplements, only 3 showed calcium deposits in the kidney, whereas 30 of 54 rats killed after the age of 90 days showed calcification in the renal parenchyma. The majority of these were killed at about 4 months of age. Rats on the stock diet rarely showed this change, and as the calcification was found in rats receiving low (B, C, D, and E) as well as high levels of supplement it was attributed to the diet rather than to hypervitaminosis D. It was found in rats bred on the stock diet and transferred to diet 30 before the 6th week as well as those bred on diet 30. In some of the rats showing calcium deposition in the renal cortex there was also calcification of the media of small arteries in the liver and elsewhere, with morphologic features suggestive of early Mönckeberg's sclerosis. There was no evidence of association of calcification with the hernias.

*Cause of Death in the Neonatal Period.* Except for the relatively small number of rats with a complete right diaphragmatic hernia, there was no gross pathologic change which explained the high neonatal mortality of the young of deficient animals. Microscopic sections of the lungs, liver, kidneys, spleen, and muscle gave no explanation of death. The majority of the deficient animals which survived to weaning and beyond had diffuse chronic bronchitis and interstitial pneumonia, but these changes were not found in the first few days of life. Observation of the living newborn rats led to the conclusion that the animals were too weak to breathe or to suckle, but they responded to painful stimuli. Whether the weakness was due to deficient function of muscle or of the nervous system was not determined.

## DISCUSSION

This series of experiments provides one more example of failure to attain the initial objective of an experiment with an incidental discovery of greater interest than the one originally sought. The general concept that congenital malformations may be produced by means of nutritional deficiency during pregnancy has been established previously. A variety of congenital malformations have been reported as the result of vitamin A deficiency by a number of authors working with various species of animals. It has been difficult to understand why the malformations reported by these workers varied so widely, especially in respect to conditions such as hydrocephalus and cyclopia, which are apparent without the aid of special technics. The present experiments suggest that species and strain differences may account for this.

In rats of the stock and D.H. strains fed on the stock diet, hernias of the diaphragm occurred in an appreciable number of cases. The incidence was increased to about 19 per cent in the stock strain bred on the deficient diet and was reduced to 2.7 per cent by means of generous supplements. In rats of the Long-Evans strain none of the control series and only one on the deficient diet was found to have a hernia. The strain differences are of a degree to be statistically significant. Nutritional deficiency therefore enhanced the degree of manifestations of this hereditary defect. The most plausible hypothesis is that in the affected strain the closure of the diaphragm was late in the strain generally, and that additional delay in the deficient animals led to an increased incidence of hernias.

Hernia of the type found in these rats is occasionally observed in the human subject but is less common than hernias through one of the natural openings of the diaphragm. It would be rash to infer that the deductions made from these experiments are directly applicable to the diaphragmatic hernia of man.

Two general concepts have arisen from these experiments which may prove to have application in the study of human neonatal mortality. The first is that a degree of specific nutritional deficiency during pregnancy may exist which is insufficient to produce obvious ill health in the mothers but which leads to death of the majority of the young; the cause of death may not be apparent on careful gross and microscopic pathologic examination. The second is that a tendency to a congenital defect may be carried as a genetic trait which is infrequently expressed under good nutritional conditions, but is frequently expressed when the diet during pregnancy lacks the appropriate specific nutritional factor. If congenital defects occur in this manner in man, it would be difficult to obtain evidence of the relationship of a specific malformation to a specific deficiency. Under general adverse nutritional circumstances in a population, however, one would expect a rise in neonatal mortality and in the incidence of congenital malformations.

#### SUMMARY AND CONCLUSIONS

A method has been devised whereby rats may be bred on a regime approximating the maximal deficiency of vitamin A compatible with reproduction. On this regime the majority of the young die during the first 2 days of life, while the mothers show few or no signs of deficiency.

No explanation of this high infant mortality has been obtained by gross examination in most cases, and microscopic examination of the lungs, liver, kidneys, pancreas, and striated muscle also failed to explain

death. No changes suggestive of cystic fibrosis of the pancreas were found.

Rats of an inbred stock albino strain which were bred on a vitamin A-deficient diet showed a high incidence of congenital diaphragmatic hernia, usually involving the posterior half of the right leaf of the diaphragm. Control animals of the same strain bred on the same diet supplemented with a small amount of haliver oil diluted in vegetable oil showed a low incidence of this malformation.

Evidence that nutritional deficiency during pregnancy was responsible for the malformation was provided by breeding rats first on the deficient diet, then feeding them a generous supplement of vitamin A and breeding them a second time. In the 9 rats of this experiment with a high incidence of the malformations in the first litters, no instances of hernia were found in the second litters.

Evidence that the deficient factor, the lack of which led to congenital diaphragmatic hernia, was in fact vitamin A was provided by two experiments: (a) Rats bred for 5 generations on the deficient diet with a supplement of haliver oil gave a low incidence of the defect, whereas the 6th generation bred without supplement gave a high incidence compared with controls of the same generation. (b) Crystalline carotene gave partial protection. The fact that it was partial was explained by the low levels of vitamin A stored in the livers of these rats, suggesting inadequate utilization of the carotene.

The tendency to diaphragmatic hernia is a genetic trait, since the defect was produced in only one rat of 111 of a second strain (Long-Evans), or 0.9 per cent as compared with an incidence of 18.9 per cent in the stock strain on the deficient diet. A substrain (D.H.) was developed from rats of the original stock strain which had a higher incidence of the defect than those of the original stock strain.

It has been demonstrated that the expression of a genetic trait may be enhanced or suppressed by means of diet during pregnancy. Evidence has been provided pointing to vitamin A as the specific nutritional factor responsible in the present experiments.

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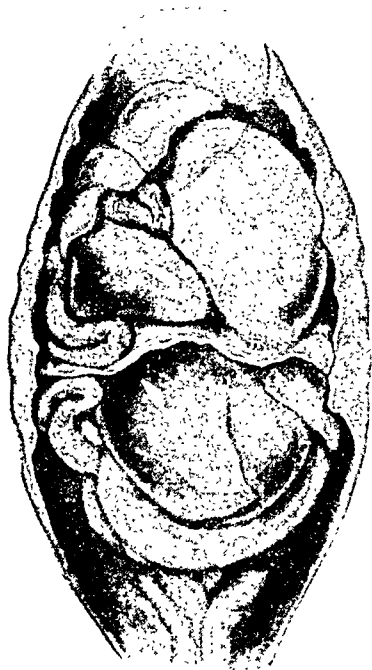
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## DESCRIPTION OF PLATE

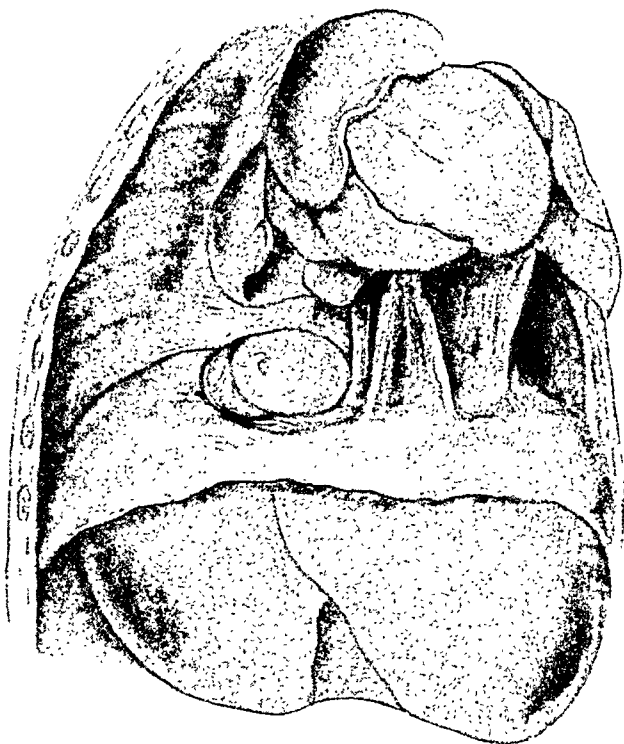
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### PLATE 23

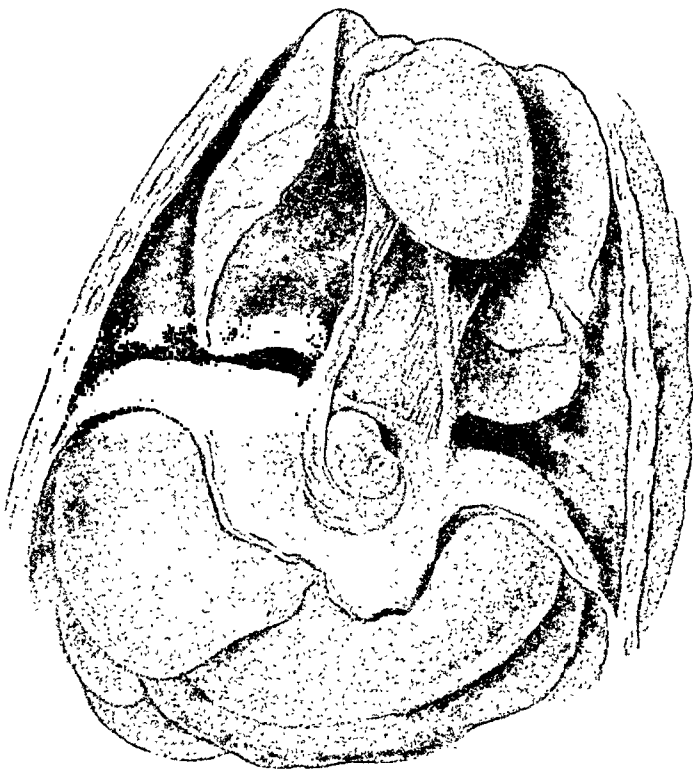
- FIG. 1. Complete right diaphragmatic hernia. The posterior half of the right leaf is absent. Portions of liver and of small intestine lie in the right pleural cavity, compressing the right lung.
- FIG. 2. Incomplete right diaphragmatic hernia. The hernial sac is a thin membrane and contains a small nodule of liver.
- FIG. 3. Pericardial hernia of the diaphragm. The sac is similar to that of the right incomplete hernia.



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## THE PATHOGENESIS OF EXPERIMENTAL HEMOGLOBINURIC NEPHROSIS IN RABBITS, WITH SPECIAL REFERENCE TO THE LATE MANIFESTATIONS \*

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The majority of studies on experimental hemoglobinuric nephrosis have placed emphasis on the reproduction of a disease in animals similar to that encountered in man. This has been accomplished with variable degrees of success.<sup>1-6</sup> Most of these studies have been concerned with the early (less than 30 days) phase of hemoglobinuric nephrosis. One study of late (more than 30 days) changes in hemoglobinuric nephrosis has been reported in which partial to complete healing occurred.<sup>6</sup> This, however, was done on only 3 dogs and the material for study was obtained by renal puncture. It is believed, therefore, that additional observations with respect to the late manifestations of experimental hemoglobinuric nephrosis in rabbits by another procedure would be desirable. This has been accomplished by performing a left nephrectomy on those animals which survived acute hemoglobinuric nephrosis. This made possible a comparison of the microscopic findings in the surgically removed kidneys with those obtained at the time of autopsy.

### METHOD

This investigation was conducted in conjunction with studies on the relationship of dehydration to the development of hemoglobinuric nephrosis, which have been reported elsewhere.<sup>5,7</sup> Rabbits were deprived of food and water for 3 days. During the next 3 days 7 to 10 intravenous injections of homologous hemoglobin equal to 1.8 gm. per kg. were given to each animal. During this period the rabbits were without food, and the only water they received was administered intravenously (22 to 38

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cc. daily). Under the conditions of this experiment approximately one-third of the rabbits died of hemoglobinuric nephrosis and two-thirds survived after variable elevations of nonprotein nitrogen. A left nephrectomy was performed under nembutal anesthesia 13 to 17 days after the initial injection of hemoglobin in those animals which survived. The operations were done during the period of declining nonprotein nitrogen. Following the nephrectomy, the rabbits were kept from 34 to 116 days and then killed. The tissues were examined and fixed in 10 per cent formalin. The kidneys were divided along the long axis. For microscopic studies, a central longitudinal block of the whole kidney was embedded in paraffin and sections were stained with hematoxylin and eosin. Since pigment casts are located principally in the cortex,<sup>5</sup> this area was selected as the most representative site. The casts in 10 low-power fields were counted and their total number was determined.

## RESULTS

### *Gross Observations*

The kidneys which were removed surgically were always congested. This was due to the clamping of the renal vessels prior to the removal of the kidney. The surgically removed kidneys were always larger than one-half the expected average combined weight of 12.5 gm.<sup>8</sup> The surface was smooth in every case. Examination of the surface revealed brown foci measuring 1 to 3 mm. in diameter in 8 of 12 rabbits. On the cut surface, it was possible to distinguish streaks of brown pigment in the cortex in 5 of 12 rabbits.

The kidneys which were removed at autopsy were heavier than those removed surgically in each case (Table I). On the cut surfaces no abnormality other than congestion of the parenchyma was evident.

There was minimal fibrosis of the myocardium in each case due to cardiac puncture for the withdrawal of blood. The lungs and spleens were normal in all rabbits.

### *Microscopic Findings*

The pertinent microscopic changes are tabulated in Table I. The total number of pigment casts observed in 10 low-power fields in the surgically removed kidneys varied from 4 to 188. They were uniformly distributed in the distal convoluted tubules or in sectors as described by Harrison, Bunting, Ordway, and Albrink.<sup>9</sup> In 3 rabbits the accumulation of pigment casts and the tubular dilatation were almost as marked as in some animals which died in uremia. It is of interest that with such extensive pigment precipitation, these rabbits were able to tolerate nephrectomy without developing uremia.

TABLE I  
*Microscopic Findings in Kidneys Following Intravenous Injections of Homologous Hemoglobin*

Rabbit	Sex	Early findings					Late findings				
		Left nephrec- tomy after hemoglobin	Weight of left kidney	Total* pigment casts	Dilated tubules	Vacuolization of tubular epithelium	Autopsy after surgery	Weight of right kidney	Total* pigment casts	Dilated tubules	Vacuolization of tubular epithelium
1	M	days 16	gm. 11.2	21	Moderate	+	days 34	gm. 12.7	4	None	—
2	F	16	7.4	4	Absent	+	34	10.2	0	None	—
3	F	16	11.8	63	Marked	+	34	12.7	0	None	—
4	F	13	9.9	40	Marked	+	47	11.8	0	None	+
5	M	13	10.1	106	Marked	+	47	14.8	5	None	—
6	F	13	8.8	35	Moderate	—	52	11.8	0	None	—
7	M	11	10.1	145	Marked	+	85	11.5	0	None	—
8	F	11	8.2	17	Minimal	—	85	12.8	0	None	—
9	M	15	7.7	40	Moderate	+	89	10.5	0	None	—
10	M	15	11.3	188	Marked	+	89	12.8	1	None	—
11	F	17		48	Moderate	—	105	6.6	1	None	—
12	M	14	8.5	8	Minimal	—	116	15.4	1	None	—

\* Sum of pigment casts seen in 10 low-power fields.

There was usually a parallelism between the number of pigmented casts and the degree of tubular dilatation. However, in 2 rabbits (3 and 4) the latter feature was more conspicuous than the former. Associated with tubular dilatation there was flattening of the epithelial cells. Local areas of necrosis of tubular epithelium were not observed in any of the rabbits which survived. It is noteworthy that even in those rabbits which died in uremia such necrosis was minimal and limited to three or four areas in one longitudinal section of the kidney.<sup>5</sup> Vacuolization of epithelial cells in the proximal convoluted tubules and in Henle's loops was present in scattered areas in 8 of 12 rabbits.

Microscopic studies of kidneys removed at autopsy demonstrated the transient nature of hemoglobinuric nephrosis in every instance. In 7 of 12 rabbits no pigment casts were found in the microscopic sections. In 5, the sections contained either isolated or a few pigment casts (Table I). In every case dilatation of tubules was no longer evident.

There was vacuolization of tubular epithelium in only one rabbit. Areas of minimal metastatic calcification of the tubular epithelium were present in 2 animals. There were a few small areas of scattered fibrosis and lymphocytic infiltration in some sections. Since these changes are known to occur spontaneously in rabbits, focal fibrosis with lymphocytic infiltration was not considered significant in this study. Examination of the kidney sections from the early and late phases failed to reveal evidence of tubular atrophy. In 3 rabbits (8, 9, and 10), 85 to 89 days after nephrectomy, there was tubular hypertrophy with regeneration of epithelial cells in the collecting tubules.

### DISCUSSION

In previous studies on the production of experimental hemoglobinuric nephrosis, the kidney lesions were always bilateral and of the same degree in both kidneys.<sup>6,7</sup> Therefore it was felt that nephrectomy performed within 13 to 17 days after injecting hemoglobin would serve several purposes. First, it would demonstrate the severity of the changes in the kidneys at the time of removal. Secondly, with knowledge of the microscopic changes, information in respect to functional kidney reserve also could be gained. When the rabbits continued to eat and gain weight after nephrectomy, the function of the remaining kidney was assumed to be adequate. It was highly instructive, therefore, to find 3 rabbits (5, 7, and 10) with moderate to extensive accumulations of pigment casts that were able to survive after the removal of 50 per cent of their functioning renal tissue.

Microscopic comparison of kidney sections removed surgically and at autopsy illustrate the transient nature of hemoglobinuric nephrosis in rabbits when the animals do not die during the acute phase. In each case practically all of the pigment casts disappeared within 34 to 116 days. Significantly enough, these animals were able to achieve complete healing even after the added burden of a left nephrectomy.

This study confirms Flink's<sup>6</sup> observations on the transient nature of experimental hemoglobinuric nephrosis, once the acute phase has passed. I saw no evidence of tubular atrophy as described by Flink. However, regeneration of epithelial cells in the collecting tubules was observed in 3 instances. This apparent discrepancy may be explained by the fact that Flink made repeated punctures to obtain material for biopsy, which undoubtedly injured some nephrons and caused subsequent atrophy of renal tubules.

### SUMMARY

When an animal survives the acute phase of hemoglobinuric nephrosis, death does not occur. Under such conditions it must be assumed that

hemoglobinuric nephrosis is a transient disease. The pigment casts, tubular dilatation, and vacuolization of tubular epithelium disappear within 34 to 116 days. Even when there is extensive deposition of pigment, tubular dilatation, and epithelial degeneration, the animals are able to tolerate still further loss of functioning renal tissue in the form of a nephrectomy.

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[ Illustrations follow ]

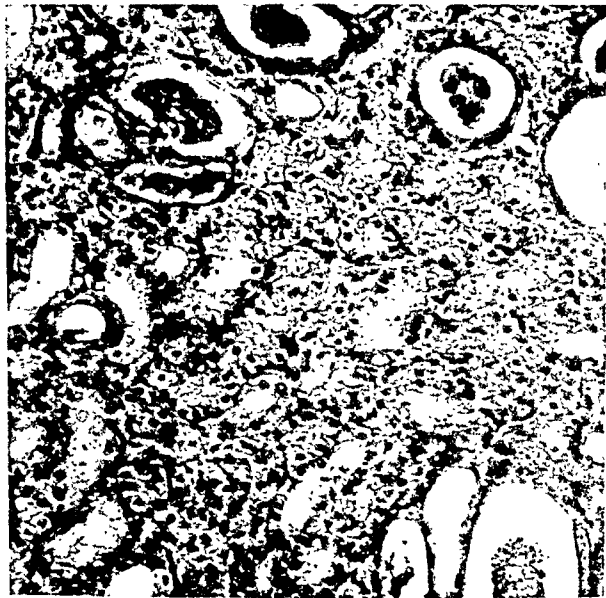
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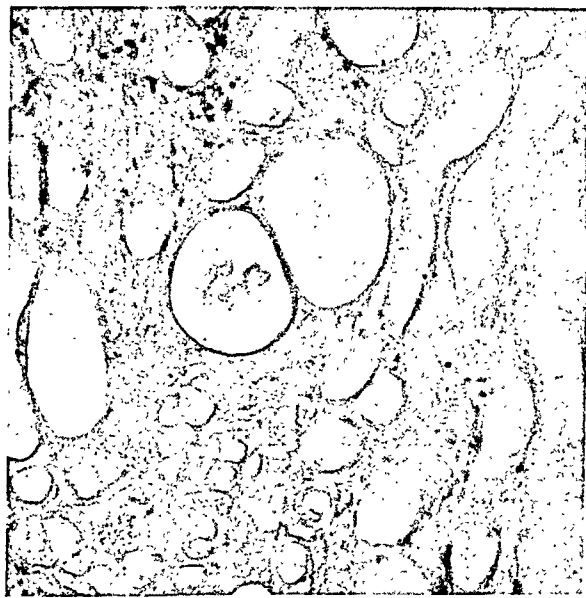
### PLATE 24

- FIG. 1. (Rabbit 7, early.) There was a total of 145 casts in 10 low-power fields, with marked tubular dilatation. The casts and tubular dilatation tended to be diffuse and were limited principally to the cortex. At autopsy 85 days later, examination of the kidney section revealed disappearance of casts and tubular dilatation. Hematoxylin and eosin stain.  $\times 150$ .
- FIG. 2. (Rabbit 5, early.) A total of 106 casts and marked tubular dilatation were found in 10 low-power fields. The involvement tended to be diffuse. The tubular dilatation was more conspicuous than the accumulation of pigment. In the kidney removed at autopsy 47 days later, only a few pigment casts remained and tubular dilatation was absent. Hematoxylin and eosin stain.  $\times 150$ .
- FIG. 3. (Rabbit 3, early.) The pigment casts and tubular dilatation occurred in sectors with uninvolved intervening areas. There was a total of 63 pigment casts in 10 low-power fields with marked tubular dilatation. Hematoxylin and eosin stain.  $\times 150$ .
- FIG. 4. (Rabbit 3, late.) No casts or tubular dilatation were evident in a section obtained 34 days after surgery. A granular eosin-staining material may be seen in some of the tubules. Hematoxylin and eosin stain.  $\times 150$ .
- FIG. 5. (Rabbit 10, early.) This rabbit had a total of 188 pigment casts with marked tubular dilatation in 10 low-power fields. The involvement tended to be diffuse. Hematoxylin and eosin stain.  $\times 150$ .
- FIG. 6. (Rabbit 10, late.) Eighty-eight days following nephrectomy, one pigment cast was observed in 10 low-power fields. There was then no evidence of tubular dilatation. Hematoxylin and eosin stain.  $\times 150$ .

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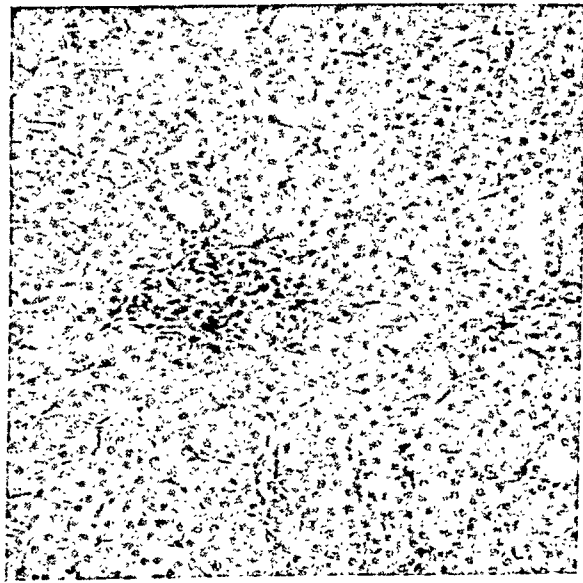
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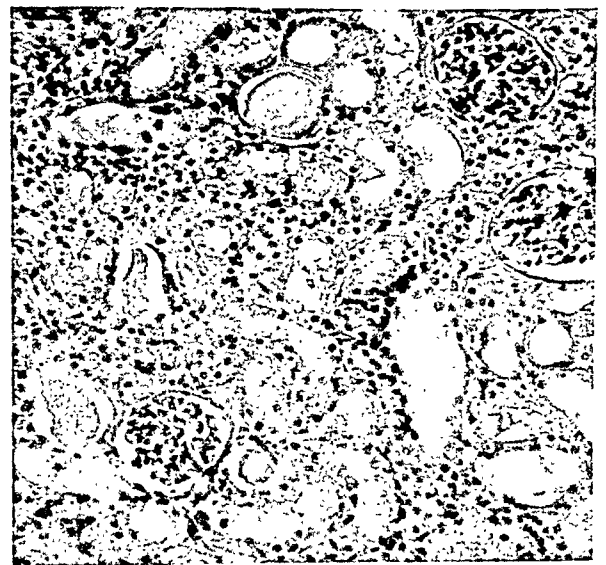
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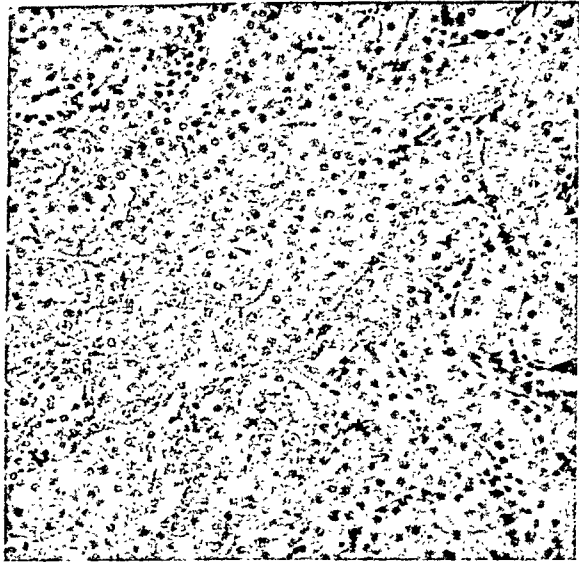
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# HEMOGLOBINURIA (BLACKWATER FEVER) IN MONKEYS A CONSIDERATION OF THE DISEASE IN MAN \*

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Although it was observed as early as 1932 that hemoglobinuria occurred in *Macacus rhesus* monkeys infected with *Plasmodium knowlesi*, apparently the mechanism by which it develops has not been established.<sup>1,2</sup> Furthermore, even with this experimental animal, the pathogenesis of blackwater fever in man has not been explained satisfactorily. Recent experimental studies on hemoglobinuria<sup>3-7</sup> and observations on acute malarial infections in ducks<sup>8-11</sup> and monkeys<sup>12</sup> have served as a stimulus to consider the problems of the mechanism of the development of hemoglobinuria in monkeys with malaria, and to consider the pathogenesis of the corresponding process in man.

In the original observations with *P. knowlesi* it was emphasized that hemoglobinuria frequently occurred when there was a severe infection.<sup>1</sup> The prerequisite, therefore, for the experimental production of hemoglobinuria is a severe infection with *P. knowlesi* in a *M. rhesus* monkey. The mortality rate is very high in this host with this plasmodium.<sup>1,2</sup> All animals do not develop hemoglobinuria; however, it frequently occurs terminally. The renal lesions accompanying a severe malarial infection in the monkey have been described.<sup>1,2,12</sup> There is general agreement in those studies that degeneration occurs in the epithelial cells lining the convoluted portion of the renal tubules, and there are occlusions within the lumina of the collecting tubules.

The recorded studies on blackwater fever in man are numerous. French physicians from 1851 to 1859 were among the first to call attention to the occurrence of dark urine in patients with malaria, although the disease had been known since the days of Hippocrates.<sup>13</sup> Thomson<sup>13</sup> considered the late recognition of this complication of malaria as due to several factors, "the most important being the exploration and development of the tropics by non-immune white races, and finally the settlement of the non-immunes in these areas for the development of trade and agriculture."

Blackwater fever is the term used to designate the occurrence of hemoglobin pigments in the urines of patients infected with malarial parasites. Hemoglobinuria may occur in a variety of conditions unasso-

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ciated with malaria.<sup>14-17</sup> It is more often associated with the more virulent forms of the disease in man, that is, with *P. falciparum* infections. This study is based upon clinical and pathologic observations made upon 10 *M. rhesus* monkeys infected with *P. knowlesi*.

### MATERIALS AND METHODS

*M. rhesus* monkeys were inoculated with *P. knowlesi*. The size and apparently the age varied widely in this group of 27 animals. Ten developed hemoglobinuria (Table I). The infection was transferred directly from donor monkeys, by intraperitoneal, subcutaneous, or intravenous inoculation of parasitized blood. Five monkeys (7, 177, 131, 29, and 124), were autopsied immediately following death. The presence of hemoglobinuria was determined by observing the passage of dark urine and by examining the urine obtained from the bladder at the time of autopsy. Blood for hematologic studies was obtained, usually from the lobe of the ear. However, it was necessary to obtain it from branches of the saphenous veins and the heart in moribund animals. Standard technics were used for the red blood cell counts. Blood smears were treated with a combination of Wright's and Giemsa's stains. The parasitemia was determined by counting the number of parasitized cells per 1000 red blood cells. The method used for the determination of carbon dioxide was that outlined by Peters and Van Slyke<sup>18</sup> for the volumetric blood gas apparatus.

The tissue used for histologic studies was fixed in Bouin's fluid. Paraffin sections were prepared and stained by the following methods: Hematoxylin and eosin, hematoxylin and phloxine, Mallory's azocarmine, and the Prussian blue stain for iron.

### EXPERIMENTAL FINDINGS

All of the monkeys, as shown in Table I, had a severe infection preceding the time of death except monkeys 227 and 12. The former was killed following a period of hemoglobinuria and the disappearance of parasites from the peripheral blood, and the latter had 300 parasitized cells per 1000 red blood cells 4 days preceding death. In a majority of these monkeys there was a marked anemia and a high degree of parasitemia at the time of the appearance of hemoglobinuria. The urine from monkey 131 was reddish brown; innumerable red blood cells were present, some being in small groups, while others formed casts. Many epithelial cells were present. The urine from monkey 7 was reddish and contained a large amount of fine, granular debris and a few epithelial cells. No red cells were observed in an uncentrifuged specimen. The

TABLE I  
Data on 10 Monkeys in Which Hemoglobinuria Occurred Following Inoculation with *P. knowlesi*

Number	Interval between inoculation and death	When hemoglobinuria occurred			Treatment	Remarks
		Interval before death	Red blood cell count	Parasitized red blood cells per 1000		
131	10 days	0	millions 1.38	324	Quinine, oxygen	Hemoglobinuria occurred before treatment began
29	6 days	24 hrs.	2.06	407	Quinine, oxygen, blood	
177	6 days	30 hrs.	2.08	346	Quinine	Hemoglobinuria stopped following second injection of quinine
227	19 days	6 days	3.06	95	Quinine, blood	
7	5 days	2 hrs.	0.95	536	None	Red blood cell count and parasite count made approximately 38 hrs. before death; hemoglobinuria occurred before quinine was given
10	5 days	14 hrs.	5.01	230	Quinine, oxygen, blood	
12	7 days	24 hrs.	1.17	84	Quinine, oxygen	Hemoglobinuria at time quinine was given
14	6 days	26 hrs.	4.01	584	Quinine, blood	
124	8 days	2 hrs.	2.67	695	Quinine, oxygen, blood	Red blood cell count made 9 hrs. before death; parasite count, 3 hrs.
151	6 days	24 hrs.	2.31	688	Oxygen	Hemoglobinuria occurred before oxygen was given

urine from the bladder of monkey 29 appeared normal. However, 24 hours had elapsed since hemoglobinuria was observed in this animal.

There is no evidence in this study to indicate that hemoglobinuria results from the administration of blood, oxygen, or quinine. Monkey 7 received none, while monkeys 10, 14, and 29 were voiding dark urine at the time quinine was first given. Monkey 151 was given oxygen following the appearance of bloody urine. Monkey 227 developed hemoglobinuria 12 days following the injection of blood, and at this time he also had malaria. The urine from this monkey appeared normal following the second injection of quinine and remained grossly normal for 4 days, during which time three more injections of quinine were administered.

Monkey 7 illustrates the rapidity with which malaria may develop, and also demonstrates the severity of the anemia that may accompany it. This monkey had a severe acidosis at the time of death, as shown below.

*Inoculated with P. knowlesi*

Days	Red blood cell count millions	Parasitized cells per 1000 red blood cells
1	5.89	0
2	5.66	0
3	5.67	18
5 8:15 a.m.	1.57	803
5 12:00 noon	0.95	536
5 1:30 p.m.	Moribund, voiding bloody urine. Killed by bleeding from heart. CO <sub>2</sub> content of plasma, 11.1 volumes per cent. CO <sub>2</sub> -combining power of plasma, 9.0 volumes per cent. Hematocrit, 0.7 per cent.	

Macroscopically, the spleen and liver in every monkey were enlarged and deeply pigmented. In fact, all tissues were pigmented. Since the pathologic changes in monkeys infected with *P. knowlesi* have been reported previously,<sup>12</sup> only the renal lesions are described here. Usually the kidneys were enlarged and deeply pigmented, and fluid exuded from the cut surface. In contrast to these changes in the kidneys of monkeys showing hemoglobinuria at the time of death, the kidneys from monkey 227, the animal that recovered from hemoglobinuria, were normal in size and color and the cut surfaces were dry. No casts were present in the kidneys of this monkey.

The glomeruli in all of the monkeys showed no significant changes. The epithelial cells lining the convoluted portion of the renal tubules always were injured. Sometimes they were so swollen that the tubular

lumina were essentially occluded (Figs. 2 and 3). In other instances, these cells were fragmented and sometimes they were desquamated into the lumina of the tubules. In the cytoplasm of many of these cells there were small masses of yellowish brown pigment, some of which stained faintly blue with the Prussian blue method for hemosiderin (Fig. 2). Larger amounts of a similar appearing pigment were present in the lumina of the renal tubules. The epithelial cells lining the loops of Henle and the collecting portions of the tubules sometimes were desquamated. This change was observed more frequently in the vicinity of large casts. The type of cast and its location within the tubules varied (Figs. 4 to 7). Some were large, homogeneous, pink-staining casts, others were formed by groups of deep-eosin-staining granules approximately the size of a red cell, while others were formed by a much smaller but similar staining granule (Fig. 5). Desquamated epithelial cells were conspicuous in some of the tubules. Amorphous and yellowish brown material also was present in the lumina of the tubules. Often a combination of the above constituents formed the casts. A large number of casts were located in the collecting portions of the tubules (Fig. 7). However, in some of the monkeys many casts were present in the convoluted portions (Fig. 5). No significant lesions were noted within the interstitial tissue. The lumina of the renal blood vessels was filled with parasitized red cells. No specific vascular lesions were noted.

### DISCUSSION

Observations in this study show that hemoglobinuria occurs in *M. rhesus* monkeys infected with *P. knowlesi*. It is interesting to note that the younger monkeys with a severe infection more often show this complication. The time of occurrence of the hemoglobinuria is influenced by the degree of parasitemia. In each instance there is a high degree of parasitemia, usually accompanied by a severe anemia. Thomson,<sup>13</sup> in studying blackwater fever in man, has emphasized the rôle of anemia. He observed "that pre-blackwater fever cases in every instance seen before the attack showed evidence of anaemia very similar in appearance to that of pernicious anaemia . . . It is obvious that the malarial anaemia before the onset of blackwater must be an important factor in the severity of the symptoms following a haemolysis, because, if the hemoglobinuria is severe in a case whose blood has been previously depleted of haemoglobin, the prognosis will be a very bad one . . . The degree of anaemia in fatal cases of blackwater is often intense and rapidly progressive."

Observations on the carbon dioxide-combining power of the plasma

were made in only one monkey. It is interesting, however, to find that in this monkey a severe terminal acidosis\* was present at the time of the hemoglobinuria. Fairley and Bromfield<sup>19</sup> observed in monkeys infected with *P. knowlesi* "the development of typical continuous acidotic dyspnoea associated with similar biochemical findings." It has been observed in ducks infected with *P. lophurae* that an increase in the carbon dioxide content of the plasma occurs with an increase in the degree of the parasitemia and the anemia.<sup>20</sup> Observations on the blood of patients with malaria for the presence of either acidosis or alkalosis apparently have been made infrequently; however, the studies of Fairley and Bromfield showed an acidosis. Thomson<sup>13</sup> found the urine to be acid in nearly every instance of blackwater fever; Burkitt,<sup>21</sup> likewise, found it acid.

At autopsy all of the monkeys had enlarged livers and spleens and the tissues were deeply pigmented. The reticulo-endothelial system was filled with pigment.<sup>12</sup> The lesions in the kidney were primarily tubular. The cells lining the convoluted portions of the tubules were severely injured, some were swollen and granular, others vacuolated, while some cells were absent, either as the result of complete degeneration or because of desquamation. No significant changes were observed in the epithelial cells either in the loops of Henle or in the collecting portion of the tubules. There were cellular debris, epithelial cells, granules of hemoglobin and hemoglobin casts within the lumina of the tubules. Apparently, the histologic changes in the kidneys of these monkeys are identical with those observed by Flink<sup>22</sup> in dogs that developed hemoglobinuria following the intravenous injection of hemoglobin. The quantity of materials within the lumina of the renal tubules in these monkeys appears to be insufficient to produce anuria in the manner suggested for cases of the so-called "lower nephron nephrosis."<sup>23</sup> Certainly, casts in the lumina of tubules may produce anuria; however, anoxia and shock appear to contribute more to the occurrence of anuria in monkeys with hemoglobinuria accompanying malaria. The dogs in Flink's experiments did not develop anuria, although there were innumerable casts in the tubules.<sup>22</sup> From the study of a case of post-transfusion uremia, Ayer and Gauld<sup>24</sup> concluded that "There is no evidence that the casts by any strictly mechanical obstruction produce either the oliguria or the epithelial necrosis." Tubular cell injury is the predominant lesion in some transfusion reactions.

\* Since the carbon dioxide-combining power was 9 volumes per cent and carbon dioxide content 11.1 volumes per cent, the alveolar carbon dioxide tension must have been abnormally high and, therefore, blood pH was probably decreased. This would represent a true acidosis.

In *P. knowlesi* infections there occurs a tremendous destruction of red blood cells by the plasmodia. A majority of this hemoglobin is converted into hematin by the parasites, and ultimately it is phagocytized by the cells of the reticulo-endothelial system. Varying amounts of hemoglobin-containing pigments from the parasitized cells are liberated either at the time the cell ruptures or following its phagocytosis. Such free pigments are removed from the blood stream by the reticulo-endothelial cells and are utilized by the host.<sup>25</sup> Hemoglobinemia has been observed by different investigators in malaria.<sup>26-28</sup> Yorke, Murgatroyd, and Owen<sup>26</sup> stated that "if we correlate these observations on the haemoglobinaemia and those on the red cell count and degree of haemoglobinuria, we find that a distinct parallelism exists. At the period of most intense hemolysis—as judged by the fall in the red cell count and the concentration and amount of haemoglobin in the urine—we find the highest degree of haemoglobinaemia." Thompson<sup>13</sup> stated "that haemoglobinemia precedes haemoglobinuria, and that in those cases where it cannot be detected it has been missed owing to its rapid elimination from the serum." Usually when hemoglobinuria occurs, there is a history of multiple attacks of malaria; however, patients may develop dark urine with their first attack of virulent malaria.<sup>13</sup> Flink,<sup>22</sup> in discussing hemoglobinuria, stated: "There is some evidence from clinical cases to support the thesis that the plasma hemoglobin level and/or the amount of hemoglobin liberated are the important factors." Bordley<sup>29</sup> reviewed 15 cases of transfusion reactions and found that the 10 patients who died received an average of 564 cc. of blood, while those that recovered received an average of only 314 cc. No patient receiving less than 350 cc. died, and none receiving more than 540 cc. recovered. Apparently there is a wide variation in the amount of hemoglobin pigments in the plasma of patients with blackwater fever.<sup>26,27,30</sup>

Yuile's<sup>7</sup> comment in 1942 on hemoglobinuria certainly seems applicable to malaria: "The literature contains a group of conflicting theories, each of which tends to be documented in a self-perpetuating fashion and few attempts have been made to explain the phenomenon of hemoglobinuria in the light of facts uncovered in other fields of renal research." Hemoglobin, when injected intravenously, is eliminated through the glomerulus and the rate is directly proportional to the concentration in the plasma.<sup>4</sup> Any increase in plasma concentration above the level at which maximum reabsorption occurs in the convoluted tubules will cause the hemoglobin to appear in the urine.<sup>4</sup> Fairley<sup>5</sup> believed that extracellular circulating hemoglobin is treated as a foreign substance which disintegrates, and the body eliminates it as soon as

possible by three major routes: (a) Absorption by the reticulo-endothelial system; (b) intravascular catabolism of hemoglobin; and, (c) by renal excretion.

Apparently there is no basic reason why extracellular hemoglobin pigments associated with a malarial infection would be eliminated in a fashion different from that which follows an intravenous injection of hemoglobin in dogs and, presumably, other mammals. The granules present in the cytoplasm of the epithelial cells of the renal tubules of the monkeys with hemoglobinuria were similar to those observed in the dog<sup>3,4</sup> following the intravenous injection of hemoglobin and in man following a transfusion reaction.<sup>29</sup> This process of storage and disintegration of the hemoglobin molecule is not unlike that seen in the liver, spleen, and lymph nodes.<sup>7</sup> Degeneration of the tubular epithelial cells, as observed in the monkeys in this study, may be the result of acidosis. Apparently, tubular lesions may occur following the intravenous injection of large amounts of hemoglobin in normal animals. Anderson and Morrison<sup>31</sup> observed tubular lesions following the intravenous injection of hematin in normal monkeys. In summarizing the pathologic findings associated with blackwater fever, Thomson<sup>13</sup> said: "These changes are what we would expect in an acidosis, and, according to Professor Bartlett, were exactly of the degree and type one gets with severe acidosis from other causes."

The results observed by Bing<sup>32</sup> in acidotic dogs injected intravenously with hemoglobin pigments apparently are identical with the changes observed in these malaria-infected monkeys with hemoglobinuria. Bing found that "The intravenous infusion of crystalline methemoglobin into dogs rendered acidotic with ammonium chloride is followed by a fall in the effective renal plasma flow, glomerular filtration rate, and the tubular reabsorptive capacity for glucose. The renal lesion in acidotic dogs infused with methemoglobin consists of hydropic degeneration of the proximal convoluted tubules, cellular necroses in the distal segment and plugging of the collecting tubules with hyaline and in some instances with pigmented casts. Dilatation of the collecting tubules and glomerular damage are absent."

The presence or absence of either oliguria or anuria was not determined in these monkeys. However, each frequently occurs in blackwater fever in man. It is significant to remember that in severe malarial infections, both in man and experimental animals, symptoms of shock may occur.<sup>12,33-35</sup> Glomerular filtration is influenced by the blood pressure, and in the presence of shock glomerular filtration is significantly decreased. Van Slyke and his group<sup>36</sup> demonstrated that a severe re-

duction occurred in the renal blood flow during circulatory failure. This was often followed by anuria and death of the animal. It is suggested that such a mechanism is more significant in producing anuria in blackwater fever than obstruction in the lumina of the tubules.

### SUMMARY

It appears that hemoglobinuria in *Macacus rhesus* monkeys infected with *Plasmodium knowlesi* and blackwater fever in man accompanying malarial infections are similar in their pathogenesis. In both there is an excess of hemoglobin pigments in the plasma resulting from either a high degree of parasitemia or a complete filling of the reticulo-endothelial cells with pigments and their inability to remove additional pigment from the blood. The circulating pigments are filtered through the glomeruli and some are absorbed by the tubular epithelial cells. Because of the presence of an excessive quantity of these pigments in the glomerular filtrate, and because the epithelial cells in the convoluted portions of the tubules are injured by the acidosis, much of the pigment that enters the tubules is eliminated through the urine. In the presence of acid urine many hemoglobin casts form within the lumina of the tubules. A varying number of epithelial cells may also be found in the lumina of the tubules, resulting from the cellular injury accompanying acidosis.

The pathologic changes in the renal epithelial cells of monkeys infected with *P. knowlesi* are similar in animals that do, and in those that do not, show hemoglobinuria. This would indicate that the lesions in the tubules do not result from the hemoglobinuria. The results are essentially the same in acidotic dogs injected with hemoglobin, in humans with a virulent malarial infection and showing hemoglobinuria, and in monkeys with a severe *P. knowlesi* infection and passing black-colored urine. This would indicate that the basic principles recently established in regard to the excretion of hemoglobin pigments by the kidney are applicable to the problem of blackwater fever in man.

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[ Illustrations follow ]

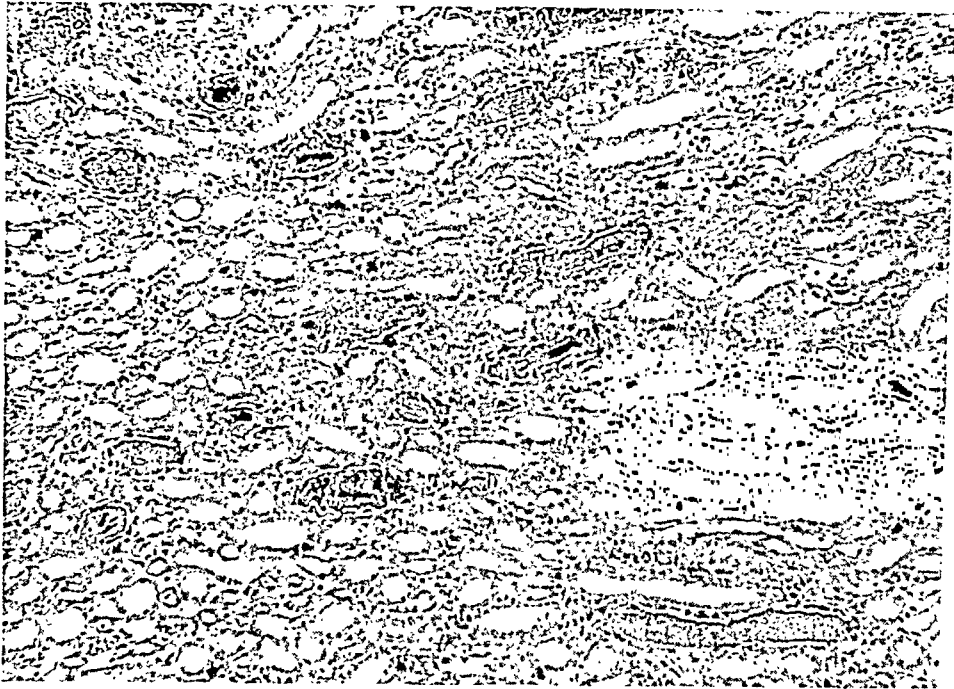
## DESCRIPTION OF PLATES

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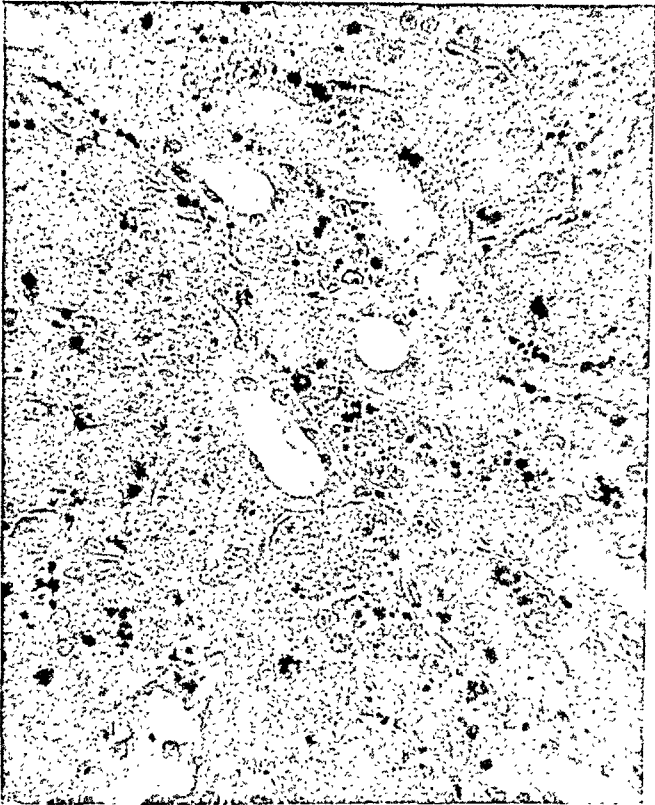
### PLATE 25

- FIG. 1. Monkey 7. A variety of casts are present in the lumina of the collecting tubules. Many of the tubules show no casts. Hematoxylin and phloxine stain.  $\times 120$ .
- FIG. 2. Monkey 7. Small granules of yellowish brown pigment are present in the cytoplasm of some of the epithelial cells. Many of the lumina are occluded by the swollen cells. Hematoxylin and phloxine stain.  $\times 300$ .
- FIG. 3. Monkey 7. The epithelial cells lining the convoluted portion of the renal tubules are markedly swollen. No significant changes are present in the glomeruli. Mallory connective tissue stain.  $\times 120$ .

1



2



3



PLATE 26

- FIG. 4. Monkey 29. Casts are present in the lumina of the convoluted portions of the renal tubules. Hematoxylin and phloxine stain.  $\times 120$ .
- FIG. 5. Monkey 29. Some of the casts are formed by pink-staining granules that vary in size, while others are pink-staining and homogeneous in consistency. Hematoxylin and phloxine stain.  $\times 300$ .
- FIG. 6. Monkey 29. Swollen and granular epithelial cells may be noted in the convoluted portion of the renal tubules. Hematoxylin and phloxine stain.  $\times 300$ .
- FIG. 7. Monkey 29. Some of the lumina of the collecting tubules are filled with casts. The epithelium in such areas sometimes has desquamated. Hematoxylin and phloxine stain.  $\times 120$ .

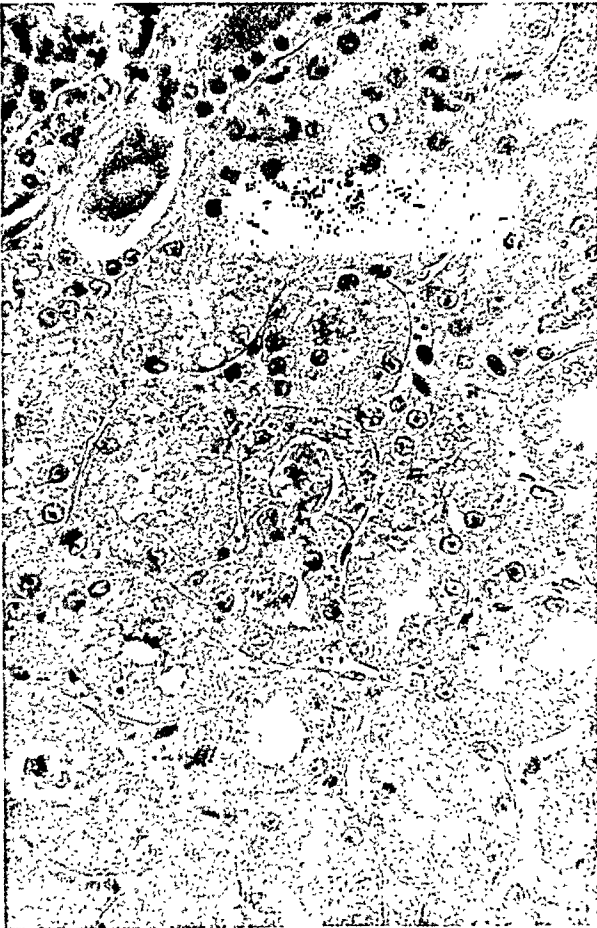
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ENLARGEMENT OF THE BRONCHIAL ARTERIES, AND THEIR  
ANASTOMOSES WITH THE PULMONARY ARTERIES  
IN BRONCHIECTASIS \*

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The double arterial blood supply of the lungs has attracted interest and discussion since its discovery. Branches from the aorta to the bronchi were known to Galen, but they were generally forgotten, and even denied by some, such as Columbo, until rediscovered by Dominico de Marchettis, and later by Ruysch.<sup>1,2</sup> There has been controversy concerning the existence of communications between the two circulations; some, like Küttner,<sup>3</sup> maintaining their presence, and others, like Cohnheim and Litten,<sup>4</sup> vigorously denying this. The confusion has been resolved to the satisfaction of most anatomists by the careful work of W. S. Miller<sup>2,5</sup> and of Ghoreyeb and Karsner.<sup>6</sup> The former, in man and dog, could find no precapillary communications between the two systems. When Miller injected the pulmonary artery with a gelatin suspension of Berlin blue, it passed through the capillaries into the pulmonary veins, but not into the bronchial arteries. When the pulmonary venous pressure was increased by clamping the pulmonary vein, there was partial injection of the bronchial artery, through the capillary networks along the respiratory bronchioles. When the injection was carried out through the pulmonary vein, the pulmonary artery was completely, and the bronchial arterial system partially, injected. When the pulmonary artery was clamped, the bronchial arterial system was completely injected from the pulmonary vein, and the pulmonary artery could then be injected from the proximal end with a contrasting material, lead chromate in gelatin. The differences in interpretation of injection experiments previous to those of Miller probably resulted from failure to correlate the results of injections with the finer anatomic detail of cleared specimens observed as three-dimensional objects. Some of the confusion resulted also from the fact that the bronchial artery is the source of the vasa vasorum for the pulmonary vessels. Furthermore, the anatomists, when dealing with human material, often failed to take cognizance of the effects of disease of the lung which may produce profound changes in the circulation. Braus,<sup>7</sup> although claiming the existence

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of anastomoses in normal material, stated that they are much more common in diseased specimens.

A most revealing study of the bronchial artery in disease has been made by Wood and M. Miller of Stanford.<sup>8</sup> They used Hill's mass (bismuth oxychloride in gum acacia).<sup>9</sup> This material was injected into the bronchial artery and roentgenograms were taken of the lungs in their inflated state. Enlargement of the bronchial arteries and numerous large anastomoses with the pulmonary artery were noted especially in subjects with tuberculosis, but also in association with chronic passive congestion, Ayerza's disease, silicosis, emphysema, asthma, and "congenital cystic disease" of the lung. One case of bronchiectasis was mentioned by Wood and Miller. In this specimen the main bronchial artery was described as being slightly dilated and quite tortuous.

In the observations to be reported it was especially in lungs showing bronchiectasis that the most notable expansion of the bronchial arterial circulation was evident. The anastomoses were of such great size and number as to suggest that they possess physiologic importance.

#### MATERIALS AND METHODS

Eighteen surgical specimens were prepared as vinylite bronchovascular casts by a method that has been described in detail elsewhere.<sup>10</sup> The bronchial arteries were identified during the course of lobectomy or pneumonectomy for bronchiectasis by their intimacy with the walls of the bronchi, tortuosity, vigorous pulsations, relatively thick walls, and content of bright red blood. They were tagged with longer sutures than the other vessels. The largest vessels were cannulated, washed with water followed by acetone, and were then injected with black plastic. After this procedure the pulmonary arteries and veins were cannulated and the lungs were inflated in a vacuum jar. Red plastic was injected into the pulmonary artery and green plastic into the pulmonary vein through tubes brought out through the cover of the jar. As this material was in the process of hardening, and while the lungs were maintained in their distended state, white plastic containing lead chromate or carbonate was introduced in a similar fashion into the respiratory tree, at first by the negative pressure within the lungs, and then under positive pressure from an injection chamber. After hardening of the plastic, sections for histologic study were cut from significant portions of the specimen. In many instances roentgenograms were made for correlation with the clinical bronchograms. Finally, digestion of the tissue was carried out in concentrated hydrochloric acid. A cast then remained of the respiratory and vascular trees.

## OBSERVATIONS

*Incidence of Enlargement of the Bronchial Arteries and Pulmonary-Bronchial Arterial Communications*

It was obvious in most instances at the time of the surgical operation that the bronchial arteries were greatly enlarged. During the process of injection of the bronchial arteries, moreover, there was usually found an abundant outflow of the black plastic from the cut ends of the pulmonary arteries at the hilum; it was never seen to issue from the veins. Since the plastic as employed for the injection was much too viscid to penetrate the capillaries, this observation at once suggested the existence of sizable anastomoses with the pulmonary arteries. When the casts were examined after injection, striking bronchial arterial enlargement and anastomoses with the pulmonary artery were found in 15 of the 18 specimens. In 11, large branches of the bronchial artery had been identified during the operative dissection or after removal of the specimen, and these were cannulated, washed, and injected directly. In 4 specimens, however, the bronchial arteries were not identified as such, until after injection of the pulmonary arteries from which they had been injected retrogradely. This was to be expected, since the reverse, injection of the pulmonary arteries from the bronchials, was commonly observed, as has been stated. The result was the same as when the arteries were injected separately, except that the objective of demonstrating the two systems in contrasting colors was not achieved. Nevertheless the bronchial vessels were easy to identify by criteria that will be detailed. An example of retrograde injection of the bronchial arteries from the pulmonary side is illustrated in Figure 1. Here the arterial system appears to be at least doubled and often many times compounded for each branch of the respiratory tree, in contrast to the simple arborization of the normal pulmonary arterial tree, where a single branch follows the course of each bronchus or bronchiole.

In 2 of the 3 specimens in which neither direct nor indirect injection of the bronchial arterial system was achieved, the bronchiectasis was of very minor degree. In the third there was severe bronchiectasis of the right middle and lower lobes but anastomoses of the bronchial and pulmonary arterial systems were not demonstrated.

*The Bronchial and Pulmonary Arteries Contrasted*

In the casts, the bronchial arteries are seen to be more intimately applied to the bronchi than the pulmonary vessels. This is to be expected since the former richly supply the mucous membrane and other elements of the walls of the bronchi themselves, while the pulmonary artery does

not yield such vessels nor any terminal branches until the respiratory bronchiole is reached.\* The pulmonary arteries pursue a rectilinear course roughly parallel to the branches of the respiratory tree. The bronchial arterial trunks, however, tend to spiral in relation to the long axis of each bronchus. The pulmonary arteries are truly end arteries and communications exist only among the finer capillary networks; on the contrary, even the larger bronchial vessels, especially in bronchiectasis, are arranged in a dense communicating network. Often the communicating branches are as large as the trunks which they unite. They resemble the arcades of the mesenteric arteries (Fig. 2). It is not rare for a bronchial artery to transgress the boundaries even of a segment. This probably results from the course of some of these arteries within the septa, where vessels from adjacent segments may meet. The plexiform arrangement of the bronchial arteries accounts for the impossibility of cutting off the systemic arterial supply of a bronchus.<sup>11</sup> So numerous are the sources of collateral supply, from branches of the subclavian, internal mammary, pericardiophrenic, esophageal, and numerous intercostal and other derivatives of the aorta, that the effect of ligating the larger identifiable bronchial arteries at their sources is always defeated by an immediate overgrowth of the accessory vessels. The major bronchi are supplied at least by pairs of vessels. Even when a compressive ligature is applied to a major bronchus, the deep mucosal vessels, or perhaps collaterals coursing within the septa or as vasa vasorum, will assure the vitality of the distal tissues.

#### *Location and Nature of the Communications Between the Pulmonary and Bronchial Arterial Systems*

An attempt was made to relate the sites of anastomosis of the two systems of vessels to the order of branching of the bronchi. For this purpose, in order to avoid ambiguity, the main segmental bronchus of each segment (in the sense of Brock,<sup>12</sup> or of Jackson and Huber<sup>13</sup> as modified by Boyden<sup>14</sup>) was considered to be of the first order, each of its first two subdivisions of the second order, and so on. No anastomoses were found proximal to the third order bronchi within the segments.† The actual observations are presented in Table I. In most instances the

\* An injection so fine as to reach these structures, although easily possible with the vinylite method, was expressly avoided by selecting 12.5 per cent material containing lamp black. With this material no vessels of a diameter less than 50  $\mu$  were injected; for the most part the injection was coarser than this.

† This is in contrast to what we have observed in congenital pulmonic stenosis. In this condition the tremendously enlarged bronchial arteries may communicate directly with the pulmonary arteries in the region of the segmental bronchi, or even of a lobar bronchus.<sup>15</sup>

anastomoses were first found along branches of the fourth order in relation to the walls of the large bronchiectatic sacs (Fig. 3). These sacculations, in 15 of our 17 cases of saccular bronchiectasis, begin either in the third or fourth orders of branching. Additional anastomoses, usually multiple and of large size, were often found far beyond the last injected sac (Fig. 1). Many of these probably occur within scar tissue; for in histologic sections it is evident that bronchi and bronchioles which

TABLE I  
*Results of Bronchovascular Vinylite Injections in Bronchiectasis*

Specimen	Mode of injection of bronchial arteries	Location of anastomoses			Maximum size of anastomoses
		Lobe	Segment	Segmental branch where first observed	
39865	Direct	R.l.l.	Basal	V	mm.
40279	Retrograde	L.l.l.	Post. basal	IV	>1.0
			Mid-basal	V	0.75
40280	Direct	L.l.l.	All	IV	<1.0
40281	Direct	R.l.l.	Ant. basal	IV	1.0
			Post. basal	V	
40282	Retrograde	L.l.l.	Mid-basal	V	<1.0
40716	Direct	L.l.l.	Basal	IV	1.0
41089	Retrograde	L.l.l.	Post. basal	V	1.0
			Ant. basal	IV	1.0
41814	Direct	L.l.l.	Post. basal	V	
			Mid-basal	V	>1.0
41855	Direct	L.l.l.	Post. basal	IV	1.0
			Mid-basal	IV	1.0
42688	Retrograde		Ant. basal	VII	1.0
42689	Direct	L.u.l.	Apical	V	
			Subapical	V	
			Lingula	IV	<1.0
		L.l.l.	Ant. basal	IV	
			Post. basal	IV	
42690	Direct	L.u.l.	All	IV	<1.0
		L.l.l.	Apical	IV	
42691	Direct	L.u.l.	All	IV	
		L.l.l.	All		<1.0
42987	Direct	R.m.l.	Lateral	VII	<1.0
		L.l.l.	All		
43651	Direct	L.u.l.	Lingula	IV	<1.0
		L.l.l.	All	IV	

branch from the dilated sacs often have minute lumina embedded within highly vascular granulation tissue, or else their lumina have become completely obliterated. Indeed it may well be that the atelectasis and fibrosis associated with this process produce the force that expands the bronchi,<sup>16</sup> and that the chief locus of the original disease is not so much in the walls of the present bronchiectatic sacs, as distal to them.

Before the actual junction with the pulmonary arteries is reached the bronchial vessels often spiral in a very remarkable fashion (Fig. 4). Spiraling of the pulmonary artery may occur also; but the coils of the

bronchial vessel are tighter, wider, and more numerous. The bronchial arteries normally pursue a spiral course about the bronchi and when fibrosis of the lung occurs, as a result of the mechanisms described, these spirals are compacted and so are more obvious.

In like manner the contraction of scar tissue from the region of the obliterated small bronchioles toward the walls of the large bronchiectatic sacs probably explains why the anastomoses often occur apparently so close to the hilum, when ordinarily the branches of both systems communicate only by the capillaries about the more distal ramifications—the respiratory bronchioles.

### *Size of the Anastomoses*

In most instances the plexuses of bronchial arteries about a single bronchus communicate at many points with the pulmonary arterial system. At each point the bronchial artery is of the same size or slightly smaller than the pulmonary twig which it joins (Fig. 5). It must be remembered that shrinkage of the vinylite occurs after injection, more in the case of the black plastic used for the bronchial vessels, which was of 12.5 per cent concentration, than of the 28 per cent "filled" material, that usually was employed for the pulmonary arteries and veins. The former shrinks an estimated 10 per cent. In 2 specimens the largest communications exceeded 1 mm. in diameter. In 5 they were  $1 \pm 0.1$  mm. in diameter and in 8 they were smaller (Table I). In the vicinity of the sacs the major bronchial arteries often approached the diameter of the pulmonary arterial trunk at the same level, and this was occasionally true even at the hilar end of a segment. In the instance illustrated in Figure 6 the relative diameters of the bronchial and pulmonary arteries were, respectively, 3.1 and 4.2 mm. These data apply only to vessels easily visible grossly. They suggest a much greater blood supply from the bronchial vessels than from the pulmonary arteries, when the greater pressure impelling the blood in the former is considered.

### *Mechanism of the Enlargement of the Bronchial Arteries*

It remains to inquire in what manner the expansion of the bronchial arterial circulation and its anastomoses is associated with the processes that lead to bronchiectasis. Necrosis with the formation of pulmonary-bronchial arterial fistulas is probably not concerned, since otherwise one would expect to find gross communications between the bronchial arteries and the pulmonary veins. But no instance of such communications was found in the abundant material that came under study (Fig. 7).

At least three changes occur during the development and after the

establishment of bronchiectasis which could not exist without an increased supply of oxygenated blood from the aorta: (1) The most significant is the organizing pneumonitis that, according to Mallory<sup>16</sup> and other observers, usually precedes the bronchiectasis (Figs. 8 and 9). At that time the newly budding capillaries that supply oxygen to the leukocytes and other elements of the granulation tissue may be derived from both systems, and these may join. Thus the peripheral capillary bed of the bronchial artery becomes markedly increased and these vessels enlarge. Similar enlargement of systemic vessels which feed large masses of granulation tissue may be observed elsewhere, and large arterial channels may persist even in a well organized scar. Since the pulmonary and bronchial trunks are immediately adjacent, it is not surprising that certain of the larger vascular channels may bring them into free communication and that these channels may persist after organization is complete. That the bronchial vessels may enlarge promptly even in acute pneumonic processes is suggested by the observations of Mathes, Holman, and Reichert<sup>17</sup> on distemper in the dog. (2) The second change is the considerable hypertrophy of bronchial smooth muscle that occurs in the walls of some of the expanded bronchi in certain cases of bronchiectasis (Fig. 10). (3) The third is the increase in lymphoid tissue which may form huge follicles, both in the walls of some of the sacs and in the proximal bronchi. An example is shown in Figure 11. A persistent large bronchial artery in a healed process is shown in Figure 12.

*Physiologic Importance of the Expanded Bronchial Circulation  
and Its Anastomoses*

The enlarged bronchial vessels which exist in the walls of the bronchiectatic sacs may be injured by bacterial agents, a frequent occurrence. The bright red blood under systemic pressure may pour into the respiratory tree with serious or even fatal consequences. A similar explanation has been given by Wood and Miller<sup>8</sup> for the large hemorrhages that may occur in some tuberculous cavities.

The anastomoses between the bronchial and pulmonary arteries account in part for the fact that there is usually little or no desaturation of the systemic arterial blood even in severe cases of bronchiectasis. The pressure in the pulmonary arteries that enter the diseased tissue is increased by their communication therein with the branches of the systemic circulation. Thus there is a shunting of pulmonary arterial blood away from the anastomoses into healthy parenchyma capable of more efficiently oxygenating the contained venous blood. In a patient recently observed at the New Haven Hospital, all parts of the left lung were

involved in bronchiectasis; the oxygen saturation of blood obtained from the radial artery was 94.4 per cent. In this patient the vital capacity was 1080 cc., and bronchspirometry showed an uptake of oxygen by the left lung of only 2 cc. per minute, while that of the right lung was 230 cc. per minute. When prepared as a bronchovascular cast, the left lung showed extreme enlargement of the bronchial arteries and numerous anastomoses of these vessels with the pulmonary arteries. Other factors concerned in shunting of blood within the lung are discussed by Hamilton, Woodbury, and Vogt.<sup>18</sup>

It is obvious that in these cases the output of the left ventricle must exceed that of the right by the amount of blood that passes in the circle: Left ventricle→aorta→bronchial artery→pulmonary vein→left auricle→left ventricle. Some conception of the magnitude of the collateral circulation that may exist in the lung under somewhat similar circumstances is gained from experimental observations on dogs after ligation of the pulmonary artery of one lung. In such animals the blood flow of the ligated side can be estimated during bronchspirometry by applying a modification of the Fick principle.<sup>19</sup> A flow of blood through the bronchial vessels in excess of 900 cc. per minute has been observed 18 months after the ligation, although normally the flow through these vessels does not exceed 27 cc. per minute.<sup>20</sup> It seems possible, from the fact that the bronchial vessels in human bronchiectasis are much larger even than those of dogs on the side of the pulmonary arterial ligation, that the collateral blood flow in the bronchiectatic lungs may well be in excess of 1 liter per minute. In dogs one might expect that this would place a burden on the left side of the heart, yet in such animals Schlaepfer<sup>21</sup> has observed hypertrophy of the right ventricle. Further quantitative studies of this subject are necessary. In patients with bronchiectasis the anastomoses open another avenue for the return of blood which reaches the lung via the bronchial arteries. In the normal lung most of the blood that enters the tissue through the bronchial arteries returns to the heart via the pulmonary veins. In many cases of bronchiectasis, however, the bronchial arteries are so large in relation to the pulmonary arteries and the anastomoses with these vessels are so numerous and wide, that it seems reasonable to suppose that there may actually be a reverse flow through some of the peripheral pulmonary arterial branches toward the heart. This hypothesis, which is based merely on anatomic observation, requires confirmation by measurement of the pressures in each of the pulmonary arteries in severe cases of unilateral bronchiectasis, and by the analysis of blood from each of these vessels. If reverse flow exists, the pressure in the proximal pulmonary

artery of the involved side should be higher and the blood richer in oxygen. If backflow does not occur, it is improbable that unilateral bronchiectasis will produce pulmonary arterial hypertension. This statement is based on the evidence brought forward by Cournand<sup>22</sup> that in man, even when the entire cardiac output is forced through a single lung after pneumonectomy, the remaining capillary bed is sufficiently adaptable so that no increase in pulmonary arterial pressure results. In severe bilateral bronchiectasis, however, when no more than a relatively small amount of intact pulmonary substance may remain, the high pressure transmitted from the region of the anastomoses may contribute to pulmonary hypertension and ultimately to the development of cor pulmonale.

#### SUMMARY

In 15 of 18 specimens of lung removed surgically from patients with bronchiectasis, and prepared as casts by the vinylite corrosion technic, great enlargement of the bronchial arteries and numerous anastomoses of these vessels with the pulmonary arteries were observed. The communications were multiple and usually occurred in the walls of the bronchiectatic sacs which involved branches of the fourth order, or more distal branches, of the segmental bronchi. In half of the specimens the anastomoses equalled or exceeded 1 mm. in diameter.

The enlargement of the bronchial vessels is associated with the development of granulation tissue during the course of the organizing pneumonia that usually precedes bronchiectasis, and with the metabolic demands of hypertrophied muscle and hyperplastic lymphoid tissue that are often observed. The anastomoses may represent persistent communicating channels, originating in the granulation tissue, that originally received vessels from both the bronchial and pulmonary arterial systems.

The anastomoses are so large and numerous as to suggest that they have physiologic importance: (a) In shunting pulmonary arterial blood away from the diseased tissue into relatively intact parenchyma, where the pulmonary blood pressure is presumably lower; (b) as a factor producing hypertension in the pulmonary circulation.

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[ *Illustrations follow* ]

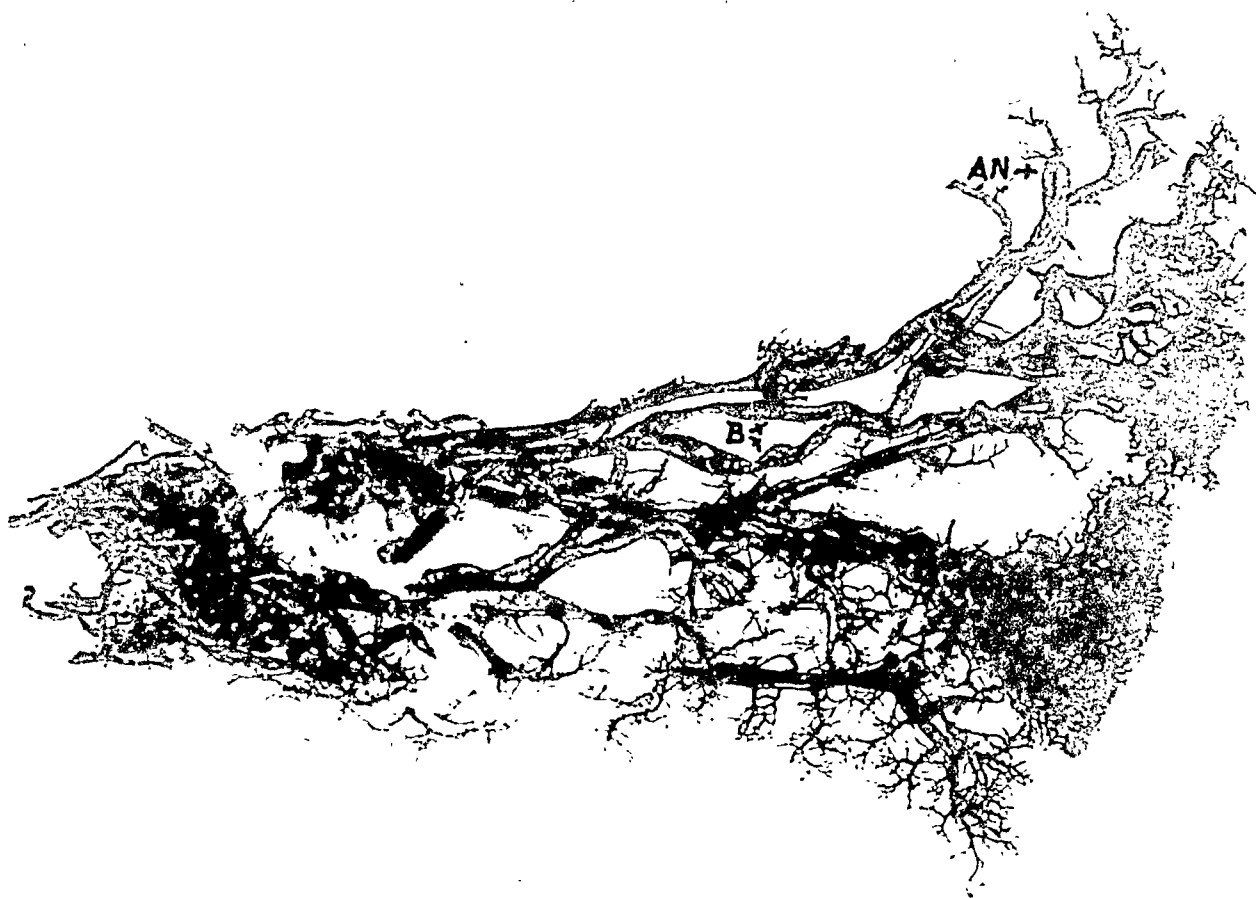
## DESCRIPTION OF PLATES

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### PLATE 27

- FIG. 1. Retrograde injection of the bronchial arterial system from the pulmonary arteries. Several branches of the pulmonary artery are shown terminating in the usual arborizing fashion. A bronchial artery (B) is seen below the uppermost pulmonary branch. The bronchial artery has a large anastomosis (AN) with this branch and with at least two other branches of the pulmonary artery.  $\times 2$ .
- FIG. 2. One large pulmonary artery (red) is distributed to each saccular bronchus (yellow) within the segment. In contrast, the bronchial arteries (black) form a dense rete in closer relation to the lumen. Multiple communications exist between the bronchial and pulmonary arteries, best seen among the uppermost branches in the photograph. The veins are injected with green plastic.  $\times 1.75$ .

1



2



PLATE 28

FIG. 3. Posterior basal segment of left lower lobe. In relation to expanded, sacular, fourth order branches of the segmental bronchus, a series of branches of the bronchial artery come into communication with branches of the pulmonary artery. Three anastomoses are clearly seen at the left in the photograph. Actually at least five are visible in the original specimen. The plexiform arrangement of the bronchial vessels again may be noted. Colors are as before.  $\times 1.5$ .

FIG. 4. Above the longest of the injected vessels is a branch of the pulmonary artery which communicates (at AN) with a bronchial artery. As the vessels are traced proximally, the latter is seen to spiral about the former and to reappear as a somewhat attenuated vessel. There has been admixture of plastic across the large anastomosis.  $\times 1.75$ .



Liebow, Hales, and Lindskog

Bronchial Arteries in Bronchiectasis

PLATE 29

FIG. 5. An anastomosis between the bronchial and a pulmonary artery is shown in relation to a bronchiectatic sac. In this instance no visible admixture of the red and black plastics has occurred across the anastomosis. The main bronchial artery is only slightly smaller than the pulmonary arteries of the same subsegment. The anastomosing branches are of approximately equal size. Of note is the more intimate relation of the bronchial artery to the lumen of the sac.  $\times 7.5$ .

FIG. 6. The large size of the main bronchial artery to a segment is shown. In this instance the bronchial artery seen below the main segmental bronchus at the left has a diameter of 3.1 mm., and the pulmonary artery of the same segment has a diameter of 4.2 mm. In its distal course the bronchial artery divides into a plexus of vessels which, in the original specimen, are injected proximally in black and distally with red plastic from the pulmonary artery by retrograde flow. In the black and white photograph the red plastic appears pale gray. Actual size.

5



6

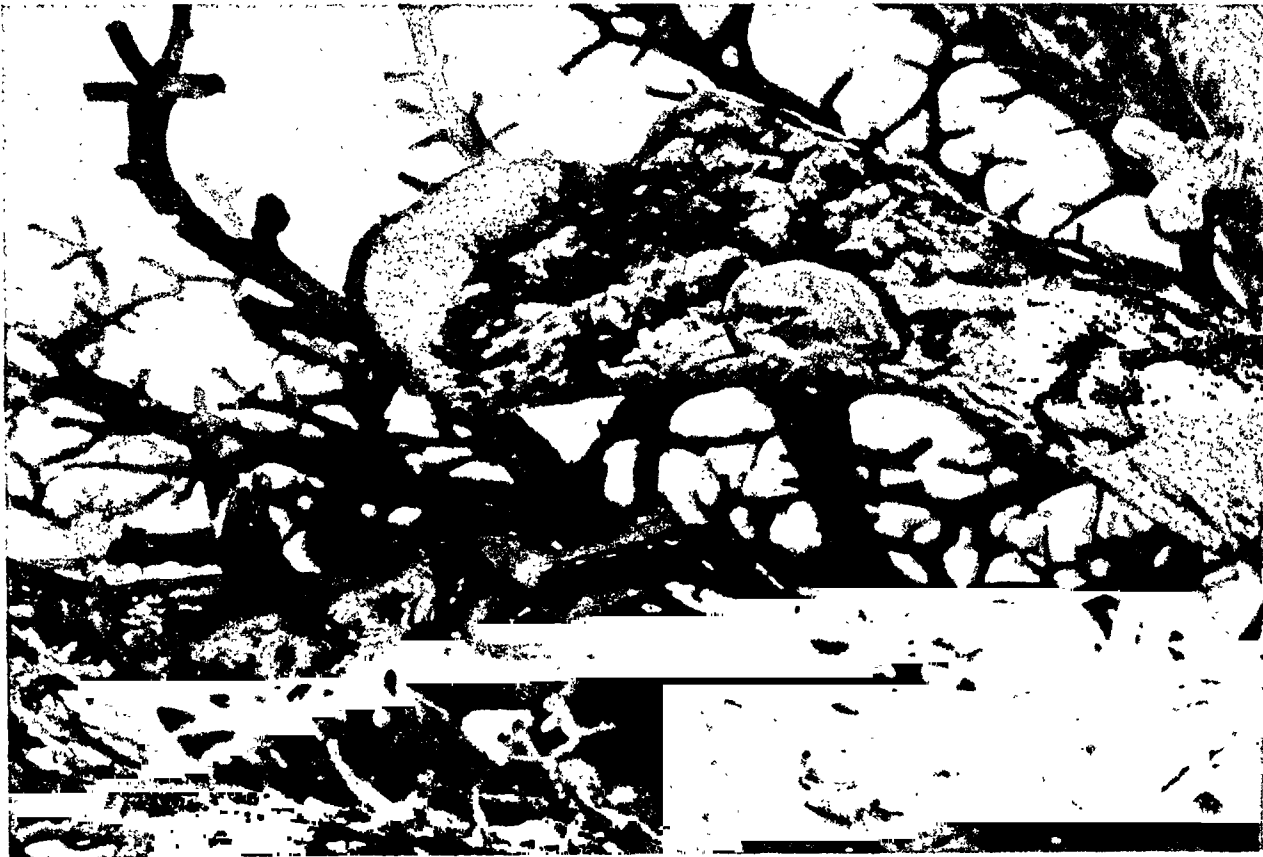




PLATE 30

- FIG. 7. The point of confluence of two bronchial arteries with a large pulmonary vessel is shown below the bronchiectatic sac. Some admixture of plastics across the anastomosis has occurred. There is no communication with the pulmonary vein (green).  $\times 4.5$ .
- FIG. 8. A large bronchial artery is seen high in the lamina propria. The trabeculations of the mucous membrane are produced by masses of highly vascular granulation tissue. These are seen under higher magnification in Figure 9. The thick wall and relatively narrow lumen of the bronchial artery may be noted. (41855.)  $\times 32$ .
- FIG. 9. Abundant vascular granulation tissue elevating the pseudostratified ciliated columnar epithelium that lines a bronchiectatic sac. (41855.)  $\times 50$ .

7



8



9



PLATE 31

- FIG. 10. Thick bundles of smooth muscle in the wall of a bronchiectatic sac. These receive their blood supply from the enlarged bronchial vessels, one of which is shown near the lower margin of the photograph. (41814.)  $\times 52$ .
- FIG. 11. Wall of a large bronchus in bronchiectasis. Large lymph follicles with prominent germinal centers are seen in the lamina propria. There is also diffuse mucosal infiltration, chiefly of lymphocytes and plasma cells. A large branch of a bronchial artery with a thick muscular wall is seen in the lamina propria. Bound to the external wall of the bronchus is a branch of the pulmonary artery. Pneumonia in process of organization involves the parenchyma surrounding the bronchus. (41910.)  $\times 32$ .
- FIG. 12. Large bronchial artery in the wall of a bronchiectatic sac. An obliquely cut branch traverses the deeper layers of the wall. (41855.)  $\times 50$ .

10



11



12





## HISTOPATHOLOGIC OBSERVATIONS IN CASES OF HODGKIN'S DISEASE TREATED WITH NITROGEN MUSTARD \*

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Several articles have been published <sup>1-9</sup> reporting the clinical response to the use of intravenous nitrogen mustard in health and neoplastic disease. The most dramatic and promising results have been obtained in diseases of the lymphatic tissues, more particularly in Hodgkin's disease. In none of these reports has there been any histopathologic detail given as to the changes in the affected lymph nodes.

The drug used at Walter Reed General Hospital has been the methyl-bis (beta-chloroethyl) amine hydrochloride. The following remarks pertain only to the use of the drug in therapeutic dosage, namely, 0.1 mg. per kg. of body weight. Occasionally, larger doses have been used, but in general this amount has been given daily, intravenously, for 4 days.

Although 55 cases (29 of Hodgkin's disease, 6 of reticulum cell sarcoma, 7 of other lymphomata, 5 of myelomata, and 8 of various carcinomas) have been treated at Walter Reed General Hospital in the past 2 years, post-treatment biopsies have not been as numerous as desirable. Nevertheless, such surgical and autopsy tissues as might be expected to indicate changes have been selected and studied repeatedly. In all, 17 cases were chosen as most likely to illustrate any consistent effect of the specific therapy. Ten of these were of Hodgkin's disease, 4 were of reticulum cell sarcoma, and there was one each of chronic lymphatic leukemia, chronic myelogenous leukemia, and transitional cell carcinoma. Sections from all 17 were studied first in unidentified grouping with other sections from cases of untreated Hodgkin's disease and lymphosarcoma, but no selection of the treated cases could be made on the basis of tissue changes.

Pre-treatment and post-treatment sections from known treated cases were then reviewed. Any suspected finding which might be attributable to nitrogen mustard therapy was checked against pre-treatment material from the same case and from untreated cases. By this means the following tentative criteria were studied: (1) The number and concentration of lymphocytes, (2) the formation of secondary follicles, (3) the presence or absence of edema, (4) the mitotic activity in the lymphocyte

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series, (5) the degree of hyperplasia of the reticulo-endothelium, (6) the development of fibrosis, (7) the degree of collagenization, (8) the mitotic activity of the reticulo-endothelium, (9) the relative number of Reed-Sternberg cells and their mitotic activity, (10) the presence of bizarre mitotic figures in any of the actively proliferating cells, (11) the state of the vessels with regard to perithelial or endothelial hyperplasia, necrosis; and obliteration, (12) the presence of cellular degeneration in lymphocytes or reticulum cells, (13) nuclear fragmentation, (14) phagocytic activity with special reference to nuclei and nuclear fragments.

In varying degrees all of these criteria were noted in treated tissues but none was found consistently in the series; in fact, no single criterion can be said to have been seen in any 2 treated cases in which that effect could be attributed to the therapy.

A single case beautifully demonstrated phagocytosis of nuclei and their fragments and is illustrated (Figs. 1 to 3). This node was removed from a case of Hodgkin's disease of sarcomatous type 5 days after a 4-day course of 10 mg. daily. Little gross shrinkage had occurred at that time but considerable shrinkage followed within the next week. However, this feature can be found in no other treated material—the nearest likeness to it is seen in pre-treatment nodes of 2 other cases (Figs. 4 to 6).

Two cases from this series have been reported<sup>8</sup> as showing degenerative changes in the reticulo-endothelial cells. After careful study of both cases we cannot confirm the reported change. Just as poorly preserved and stained reticulo-endothelial cells can be demonstrated in untreated material. In animal experiments heavy dosage produced no such change.<sup>9-11</sup>

Most of the criteria used in this study have been variously reported as having been found following roentgen-ray therapy<sup>12</sup> and in animals rather heavily treated with the various "mustards."<sup>9-11,13-15</sup>

All workers agree that the lymphocytes disappear earliest from the circulating blood after nitrogen mustard therapy and that their loss in the lymph nodes follows promptly. The established short life of the lymphocyte of 12 hours or less,<sup>15,16</sup> with a reserve lower than that for the leukocytes and erythrocytes, would appear to account for this result. There is also a suppression of the activity of that reserve by direct action of the drug upon the depots in the lymph nodes.<sup>9</sup> We have recently seen the shrinkage response of lymph nodes delayed for several days in a case of lymphatic leukemia while the pre-treatment white count of 136,100 dropped to about 5,000. Another observation in this same case, in a node removed after 1 day of therapy, was an apparent decrease in the amount of intercellular fluid.

We have attempted to find the critical period when there might be a wave of lymphocyte destruction, by securing lymph nodes during the treatment, immediately following the 4-day course and at varying later intervals. It has been estimated that a noninjurious quantity of nitrogen mustard remains in the blood stream at the end of 15 minutes.<sup>11</sup> Functional changes, therefore, may be expected promptly but demonstrable morphologic change may not occur for many hours.<sup>14</sup>

The prolonged clinical remissions in some cases are difficult to understand in the absence of any demonstrable microscopic changes after therapeutic doses. In fact, it is difficult to rationalize the use of a drug, which primarily attacks the lymphocytes, in the treatment of a disease generally considered to be one of the reticulo-endothelium. It is fully recognized that the variegated patterns of Hodgkin's disease, which may vary even from node to node in the same patient, make evaluation of fine changes in detail a difficult problem. It is also recognized that the material studied is small in numbers. It would appear that so powerful an agent, yielding such striking clinical results, even though usually transient, should have been controlled by closer histopathologic study. If such studies were made, their omissions from the published reports would imply a series of inconclusive or negative findings similar to ours.

The experimental results in animals, recently released for open publication, all include descriptions of quite definite histopathologic changes, but it must be remembered that the doses were generally large and the response was in other than human tissue. Such results must not be inferred as occurring in the human patients being treated with much less toxic therapeutic doses. It is with this warning idea in mind and in the hope that further observations on human material may be recorded, that the present brief report is submitted.

We wish to acknowledge the assistance of Capt. E. M. Greenspan, M.C., whose clinical studies were made available to us.

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\* Search of the current literature fails to find open publication to date. "PB" number refers to the file identification at the Office of Technical Services, U. S. Dept. of Commerce, Washington 25, D.C. Photostatic or microfilm copies may be obtained from that source or The Army Medical Library. A full list of medical reports now released from the "classified" list is given in the Current List of Medical Literature, Vol. 12, No. 7-B, of February 28, 1947.

## DESCRIPTION OF PLATE

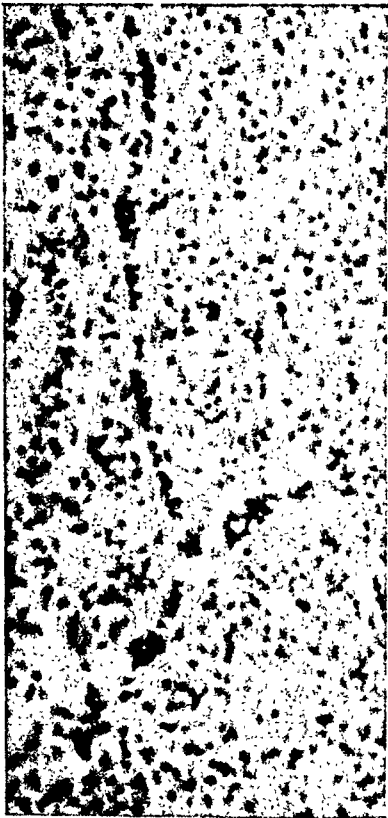
### PLATE 32

FIGS. 1 and 2. Lymphatic channels in a node with Hodgkin's disease, showing phagocytosis of lymphocytes and their nuclei 5 days after a course of nitrogen mustard.  $\times 140$ .

FIG. 3. Lymphocytic phagocytosis in the same node at a higher magnification.  $\times 750$ .

FIG. 4. The same phenomenon as in Figures 1 to 3, but from a node of an untreated case of Hodgkin's disease.  $\times 140$ .

FIGS. 5 and 6. Lymphocytic phagocytosis in the same node used for Figure 4.  $\times 750$ .



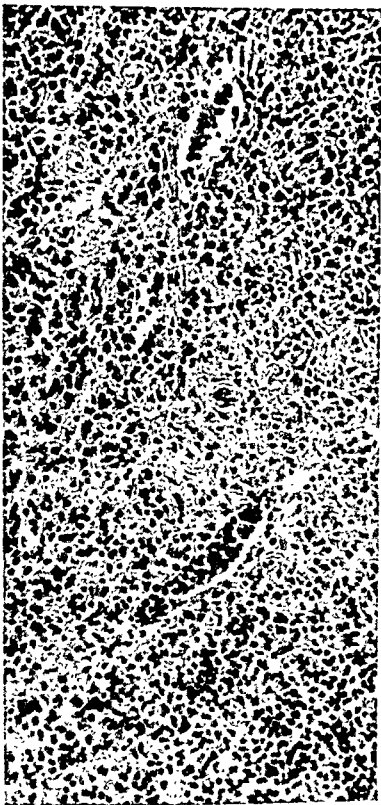
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6



# CELLS OF THE MEGAKARYOCYTE SERIES IN PERNICIOUS ANEMIA: IN PARTICULAR, THE EFFECT OF SPECIFIC THERAPY \*

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Few observations have been published of the morphologic characteristics and numbers of megakaryocytes in the bone marrow of patients with pernicious anemia. Gáspár <sup>1</sup> (1926) found in such patients that the number of megakaryocytes was slightly diminished and that certain degenerative changes occurred in the individual cell. According to this author, the nucleus first lost its fine chromatin network and stained more intensely. It then became somewhat angular and still later stained in a homogenous manner. Next, the nucleus changed into spherical forms of different sizes or into "formlosen" pieces, but remained joined in one mass. Finally, the nucleus seemed to disappear completely. As the nucleus became angular the cytoplasmic granules stained less intensely and then progressively lost their color until they became imperceptible. Fontana <sup>2</sup> (1928) stated that megakaryocytes were extremely rare and even absent in some cases. Tempka and Braun <sup>3</sup> (1932) described megakaryocytes consisting of nuclei for the most part devoid of cytoplasm. Segerdahl <sup>4</sup> (1935) found the number of megakaryocytes to be diminished, and Dameshek and Valentine <sup>5</sup> (1937) also stated that they were decreased in number or completely absent in the bone marrows of patients with pernicious anemia. These authors did not describe any qualitative changes.

Jones <sup>6</sup> (1938) described the megakaryocyte of pernicious anemia as having a coarse, polymorphous nucleus and an intensely basophilic cytoplasm, devoid of azurophilic granules and having streaks of hyaloplasm and irregular areas of spongioplasm. Rohr <sup>7</sup> (1940) found decreased numbers of megakaryocytes in the marrows of patients with pernicious anemia. These cells were described by him as being larger than normal, with marked nuclear segmentation and mature, but still basophilic, cytoplasm which contained few granules. Japa <sup>8</sup> (1943) described normal megakaryocytes as multinuclear cells which develop from unicellular sources by repeated, mitotic nuclear division without cytoplasmic division. The author classified the megakaryocytes on the basis of the number of nuclei present in the cells. He described five groups with 2, 4, 8, 16, and 32 nuclei occurring in frequency in the respective percentages of 2.5, 25.5, 53.0, 18.0, and 1.0. In a case of pernicious anemia he found

\* Received for publication, April 29, 1948.

a relative increase in the number of megakaryocytes having 2 and 4 nuclei and a relative decrease in the number of cells having 8, 16, and 32 nuclei. Paseyro<sup>9</sup> (1945) also described increased segmentation, without separation, of the megakaryocyte nuclei in cases of pernicious anemia.

#### METHOD, MATERIAL, AND DEFINITIONS

The opportunity of studying the bone marrow of a small group of patients with pernicious anemia before and after clinical and hematologic remissions has recently been available. Differential megakaryocyte counts revealed certain morphologic and numerical changes in the cells of this series, as reported in the present communication.

Five patients were studied, all of whom had clinical and hematologic changes typical of pernicious anemia. Cases 1 and 2 had been on initially ineffectual therapeutic regimens for 20 and 24 days, respectively. Thereafter, 15 U.S.P. units of purified liver extract were administered intramuscularly daily and a characteristic remission was induced in both patients. Case 3 received pteroyldiglutamylglutamic acid, 6.4 mg. daily intramuscularly (containing 4.0 mg. of potential pteroylglutamic acid) for a 14-day period, with a slight reticulocyte response. From the 9th to the 16th day, 15 U.S.P. units of purified liver extract were given daily in addition to the pteroyldiglutamylglutamic acid. This resulted in a satisfactory clinical and hematologic response. Case 4 received 15 U.S.P. units of purified liver extract every second day for a 10-day period and twice weekly thereafter for the next 3 weeks. Case 5 was given 15 U.S.P. units of purified liver extract daily for 7 days, then every second day for 10 days, and twice weekly for 2 weeks. Cases 4 and 5 responded in typical fashion.

Counts of the platelets in the peripheral blood were made with a modification<sup>10</sup> of the direct method described by Rees and Ecker.<sup>11</sup> In my experience, the range of normal variation has been from 150,000 to 400,000 per cmm. Usually two specimens of bone marrow were obtained by aspiration from each patient. The first was taken before treatment was begun; the second, after the reticulocyte response had occurred and the numbers of platelets in circulation had increased. In case 1, the first marrow specimen was obtained after transfusion of the red cells from 1000 cc. of blood-bank blood, but before liver extract was administered. Approximately 0.2 cc. of marrow fluid was aspirated from the sternal marrow cavity with a Turkel needle and small portions of the fluid immediately spread on glass slides. These were permitted to dry in air and later were stained with Wright's stain. The method described by Dameshek and Miller<sup>12</sup> for estimating the number of megakaryo-

cytes in the marrow was employed. This involves counting the number of megakaryocytes present in an area of the preparation and expressing the result in terms of a million nucleated cells. The count is facilitated by accurately determining the number of nucleated cells in 20 oil-immersion fields.

Studies of the marrows obtained before treatment revealed many multinucleated as well as mononuclear cells of the megakaryocyte series. The term *polykaryocyte* has been applied to cells of the megakaryocyte series that contain multiple nuclei in a single cytoplasmic mass. For the identification and classification of these multinucleated cells the following nomenclature and criteria were used.

*Young Polykaryocyte.* A young polykaryocyte is a multinucleated cell containing 2 or 4 nuclei (Fig. 1). These nuclei have a fine chromatin network and may occasionally contain a nucleolus. The deeply basophilic cytoplasm is homogenous in appearance and may have a few purplish red cytoplasmic granules. Occasionally, portions of the cytoplasm of these cells appeared to be in the process of detachment, possibly in the formation of platelets.

*Intermediate Polykaryocyte.* An intermediate polykaryocyte may have from 2 to 8 nuclei, but usually has 4 (Fig. 2). The nuclear chromatin is more densely clumped than in the young polykaryocyte, giving it a porous or latticework appearance. Nucleoli are not seen. The cytoplasm is lightly basophilic and may contain from a few to a moderate number of fine purplish red granules. These cells frequently appeared to be forming platelets.

*Mature Polykaryocytes.* The mature polykaryocyte usually has 6 to 10, occasionally 4 to 18, nuclei (Fig. 3). The chromatin of the nucleus is somewhat more dense and compact than that of the intermediate polykaryocytes. Nucleoli are absent. In some instances 2 or 3 of the nuclei appear to be grouped or fused together while the remainder are distinctly separated in other parts of the cell, as shown in Figure 4. The cytoplasm most often has a neutral or buff color but may at times show a trace of basophilia. Numerous granules similar to those described above are present and platelets appear to be in process of formation by these cells.

The size of the polykaryocytes increases as the cells apparently become more mature. In general, these cells are somewhat larger than the mononuclear megakaryocytes at the same level of maturation.

For the identification and classification of the mononuclear cells of the megakaryocyte series, the following nomenclature and criteria were employed.

TABLE I

*Differential Counts by Percentage and Total Numbers of Megakaryocytes*

Case no.	Date	Blasts	Mononuclear megakaryocytes									Young	
			Pro-		Lymphoid		Intermediate		Adult		Total		
			Platelet formation		Platelet formation		Platelet formation		Platelet formation				
			Present	Absent	Present	Absent	Present	Absent	Present	Absent		Present	Absent
1	12-21-46*		3.6		2.4		12.0		2.4	14.4	34.8		
	2-26-47		17.6	1.6	3.2	3.2	8.0	6.4	19.2	16.0	75.2		
2	12-26-46*		7.2		1.2		6.0		4.8	7.2	26.4		
	1-29-47				36.4				36.4	18.2	91.0		
	2-26-47		1.9	1.9	3.8	3.8	24.7		3.8	41.8	81.7		
3	11-29-46*		1.8	1.8	9.0	1.8	12.6				27.0		1.8
	12-24-46		17.6	2.2	4.4	6.6	11.0		13.2	26.4	81.4		2.2
	1-21-47		8.4	12.6	4.2	8.4	4.2	21.0	25.2	12.6	96.6		
4	1-25-47*		41.5		8.3						49.8		
	2-24-47		18.2		1.3	7.8	14.3	16.9	37.7	96.2			
5	4-4-47*		2.5	5.0	2.5		7.5		2.5	12.5	32.5	2.5	
	4-24-47		30.0				5.0	20.0	10.0	25.0	90.0		

\* Initial count made prior to the administration of therapy. The remaining counts were made

**Megakaryoblast.** Megakaryoblasts usually are 20 to 35  $\mu$  in diameter. The nucleus usually is round or oval. It has a fine chromatin structure and frequently contains several nucleoli. The basophilic cytoplasm does not contain granules and the cell shows no evidence of platelet formation.

**Promegakaryocyte.** The promegakaryocyte usually is somewhat larger than the megakaryoblast, although it may be of approximately the same size. The nucleus is oval or partially lobulated. Its chromatin is more clumped than in the megakaryoblasts and nucleoli are not seen. Only a small amount of cytoplasm is present relative to the size of the nucleus. The cytoplasm often contains a few azurophilic granules and in some instances apparent platelet formation may be found at the periphery of the cell.

**Lymphoid Megakaryocytes.** The size of the lymphoid megakaryocyte usually is somewhat greater than that of the promegakaryocyte. The nucleus is lobulated and relatively small compared to the amount of cytoplasm present. Its chromatin is dense and nucleoli are absent. The cytoplasm is basophilic and usually does not contain granules. Only rarely do these cells seem to be producing platelets.

*and Polykaryocytes in the Bone Marrow of 5 Cases of Pernicious Anemia*

Polykaryocytes					Degenerated forms		Mitotic figures	Total number of mononuclear megakaryocytes and polykaryocytes per million nucleated cells	Total percentage with platelets	Range of platelet counts in peripheral blood (in thousands)
Intermediate		Mature		Total	Vacuolated	Smudge				
Platelet formation		Platelet formation								
Present	Absent	Present	Absent	Total	Vacuolated	Smudge				
	21.6		36.0	57.6	1.2	8.4		178	2.4	26-68
	1.6	1.6	8.0	11.2		12.8		259	49.6	213-280
4.8	28.8	3.6	25.2	62.4		7.2	1.2	442	20.4	136-190
						9.1		91	36.4	219-281
	5.7		1.9	7.6	1.9	9.5		157	9.5	260-280
	23.4	5.4	37.8	68.4	5.4			670	16.2	54-71
	4.4		2.2	8.8	4.4	6.6		361	37.4	140-248
	4.2			4.2				287	42.0	192-293
8.3		24.9	16.6	49.8				128	83.4	76-98
						5.2		287	42.9	170-230
5.0	30.0	5.0	15.0	57.5	2.5	7.5		147	25.0	66-94
						10.0		100	45.0	200-300

after the institution of anti-pernicious anemia therapy.

*Intermediate Megakaryocyte.* The intermediate megakaryocyte is considerably larger than the promegakaryocyte. The nucleus contains dense chromatin and shows varying degrees of lobulation. Many granules are present in the lightly basophilic cytoplasm. Intermediate megakaryocytes often appear to form platelets.

*Adult Megakaryocyte.* The adult megakaryocyte is a large cell, usually well over 40  $\mu$  in diameter. Its multilobed nucleus has dense, compact chromatin. The abundant, neutrophilic or slightly basophilic cytoplasm contains numerous azurophilic granules. These granules form into groups surrounded by nongranular cytoplasm at the periphery of the cell. Pseudopodial processes containing these platelet-like bodies are often seen.

Listed under the heading of *smudge* (Table I) are naked megakaryocyte nuclei or remnants of nuclei and cytoplasm that had been damaged during the preparation of the smear. Fragments of cytoplasm occurring without nuclei were not included in these counts.

It is to be emphasized that in the examination of bone marrow one sees numerous transitional forms of all series. Frequently, therefore, an



individual polykaryocyte or mononuclear megakaryocyte may not rigidly conform to the description of the cell type at a particular level of maturation. Such a classification as that described here and elsewhere is of value, however, in that it permits a grouping of cells of approximately the same stage of development for comparison of counts obtained serially from a given patient and those obtained from different sources.

### RESULTS

The results of the counts of the cells of the megakaryocyte series are given in Table I. The most significant findings are the changes in the ratios of polykaryocytes and mononuclear megakaryocytes as remissions were induced by liver extract therapy. Before remission, the percentages of polykaryocytes were invariably high, ranging from 49.8 to 68.4, but this increase bore no relation to the initial hemoglobin levels, which were between 4.1 and 10.1 gm. per 100 cc. of blood. In remission only 0.0 to 11.2 per cent of polykaryocytes were present. Conversely, prior to treatment, the percentages of mononuclear megakaryocytes were low, with extremes of 26.4 and 49.8, and in remission were high, varying from 75.2 to 96.6. These changes applied to the absolute as well as to the relative numbers of polykaryocytes and mononuclear megakaryocytes.

In 4 of the 5 patients, the increased number of polykaryocytes found in the initial examination of the bone marrow was associated with low platelet counts in the peripheral blood. In the fifth patient, the platelet counts were in the lower range of normal variation. In each instance as the patient responded to therapy and the platelet counts in the peripheral blood increased, the number of polykaryocytes in the marrow decreased. The total numbers of mononuclear megakaryocytes and polykaryocytes obtained by the initial marrow aspiration varied from 128 to 670 cells per million nucleated marrow cells. Three of these five initial counts were within the normal range of 99 to 270 megakaryocytes per million cells established by Dameshek and Miller,<sup>12</sup> while the remaining two counts exceeded this range. The megakaryocyte counts that were within the range of normal were obtained when there was peripheral thrombocytopenia, and one of the elevated megakaryocyte counts was obtained when there was no thrombocytopenia. In 3 of the 5 cases the percentage of cells of the megakaryocyte series exhibiting apparent platelet formation was less in the initial specimen obtained before treatment than subsequently when remission had occurred. This correlated well with the change in the platelet counts in the peripheral blood. However, in case 2, when the patient was in clinical and hematologic remis-

sion, only 9.5 per cent of the total number of the cells of the megakaryocyte series appeared to be producing platelets. In case 4, when there was peripheral thrombocytopenia, 83.4 per cent of the megakaryocytes apparently were forming platelets; subsequently when remission had occurred and the platelet counts were normal, only 42.9 per cent of the megakaryocytes appeared to be forming platelets. Thus, in these studies there seemed to be no constant relationship between the platelet counts in the peripheral blood and the total number and percentage of cells of the megakaryocyte series apparently producing platelets.

### DISCUSSION

Di Guglielmo <sup>13</sup> originally expressed the view that the polykaryocyte is an intermediate cell type that develops during the formation of the mature megakaryocyte from fusion of primitive mononuclear cells. This interpretation of the origin of the adult cells of this series is a point disputed by hematologists. Although polykaryocytes are observed occasionally in specimens of bone marrow obtained from normal children and adults, and in the marrows of patients having a variety of diseases of the hematopoietic tissues, they are not of the type of megakaryocyte usually seen.

There is little possibility of confusing the polykaryocytes described above with osteoclasts and multinucleated immature cells of the erythrocyte series. Not only do they have different morphologic characteristics of the nucleus and cytoplasm,<sup>14</sup> but also the cytoplasm of the polykaryocyte contains granules and at times appears to produce platelets, while cells of the other two types do not. Nor can polykaryocytes be considered to be degenerating megakaryocytes since they appear actively to form platelets and do not exhibit features of degeneration, such as vacuolization or hyalinization of the cytoplasm or pyknosis of the nucleus.

In the marrow of the cases of pernicious anemia studied, young, intermediate, and adult forms of polykaryocytes and mononuclear megakaryocytes apparently coexisted. Before treatment the polykaryocytes were present in relatively increased numbers, while the converse situation existed in remission. The data presented above indicate that before treatment the increase in the number of polykaryocytes is independent of the hemoglobin concentration in the peripheral blood.

Furthermore, the opportunity was presented by Dr. Charles S. Davidson to review bone marrow preparations made before and after transfusions of whole blood and of red cell concentrates as well as after specific liver extract therapy in patients with pernicious anemia. David-

son, Murphy, Watson, and Castle<sup>15</sup> found that, entirely without specific therapy, transfusions sufficient to elevate the erythrocytes and hemoglobin to normal values caused the disappearance of the megaloblasts from the bone marrow in pernicious anemia, presumably as a result of the elevation of the oxygen-carrying power of the blood. However, the numbers of leukocytes and platelets in the peripheral blood were not increased and examination of the bone marrow smears revealed no significant reduction of the numerous polykaryocytes present before transfusion. Indeed, these cells became decreased or were completely absent only after adequate specific therapy, in the form of liver extract, had been administered. Thus, in contrast to the situation with respect to the megaloblasts, it seems that in pernicious anemia the mononuclear megakaryocyte:polykaryocyte ratio is not readily modified by increase in the oxygen-carrying capacity of the peripheral blood. Instead, it depends on the presence of the anti-pernicious anemia factor(s), the administration of which also diminishes the megaloblasts in the marrow in pernicious anemia prior to significant increase in the oxygen-carrying capacity of the blood.<sup>16</sup>

With the technic of study employed, there was no evidence of decreased numbers of cells of the megakaryocyte series in the marrows of patients before treatment. Indeed, in 2 of the 5 cases the numbers of these cells exceeded the range of normal established by Dameshek and Miller.<sup>12</sup> In the remaining 3 cases the megakaryocyte counts were normal.

### CONCLUSIONS

Repeated bone marrow studies of 5 cases of pernicious anemia have been made with emphasis on the morphologic appearances and numbers of mononuclear megakaryocytes and polykaryocytes.

Before remission was induced by specific therapy there was an increased number of polykaryocytes and a decreased number of mononuclear megakaryocytes in the bone marrow, while following remissions induced with liver extract, this ratio of cells was reversed.

Evidence is presented to indicate that, in contrast to the situation with respect to the young erythrocyte-forming cells (megaloblasts), a shift in the type of bone marrow megakaryocyte does not occur with an artificial increase in the oxygen-carrying capacity of the peripheral blood as a result of transfusions given without the administration of specific therapy.

A decrease in the percentage of bone marrow polykaryocytes, however, does follow the administration of adequate amounts of anti-pernicious anemia therapy as does also the disappearance of megaloblasts

prior to a significant increase in the oxygen-carrying capacity of the peripheral blood.

The total number of cells of the megakaryocyte series was either normal or increased in the marrows of patients in relapse.

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[ Illustrations follow ]

## DESCRIPTION OF PLATES

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### PLATE 33

- FIG. 1. "Young" polykaryocyte from case 5. This cell has 4 nuclei containing fine chromatin, and a basophilic cytoplasm in which there are a few scattered granules. A few platelets apparently are being formed at one portion of the periphery.  $\times 1150$ .
- FIG. 2. "Intermediate" polykaryocyte from case 1. The chromatin in these 7 nuclei shows increased clumping. The faintly basophilic cytoplasm contains many small azurophilic granules. There is no evident platelet formation.  $\times 1150$ .

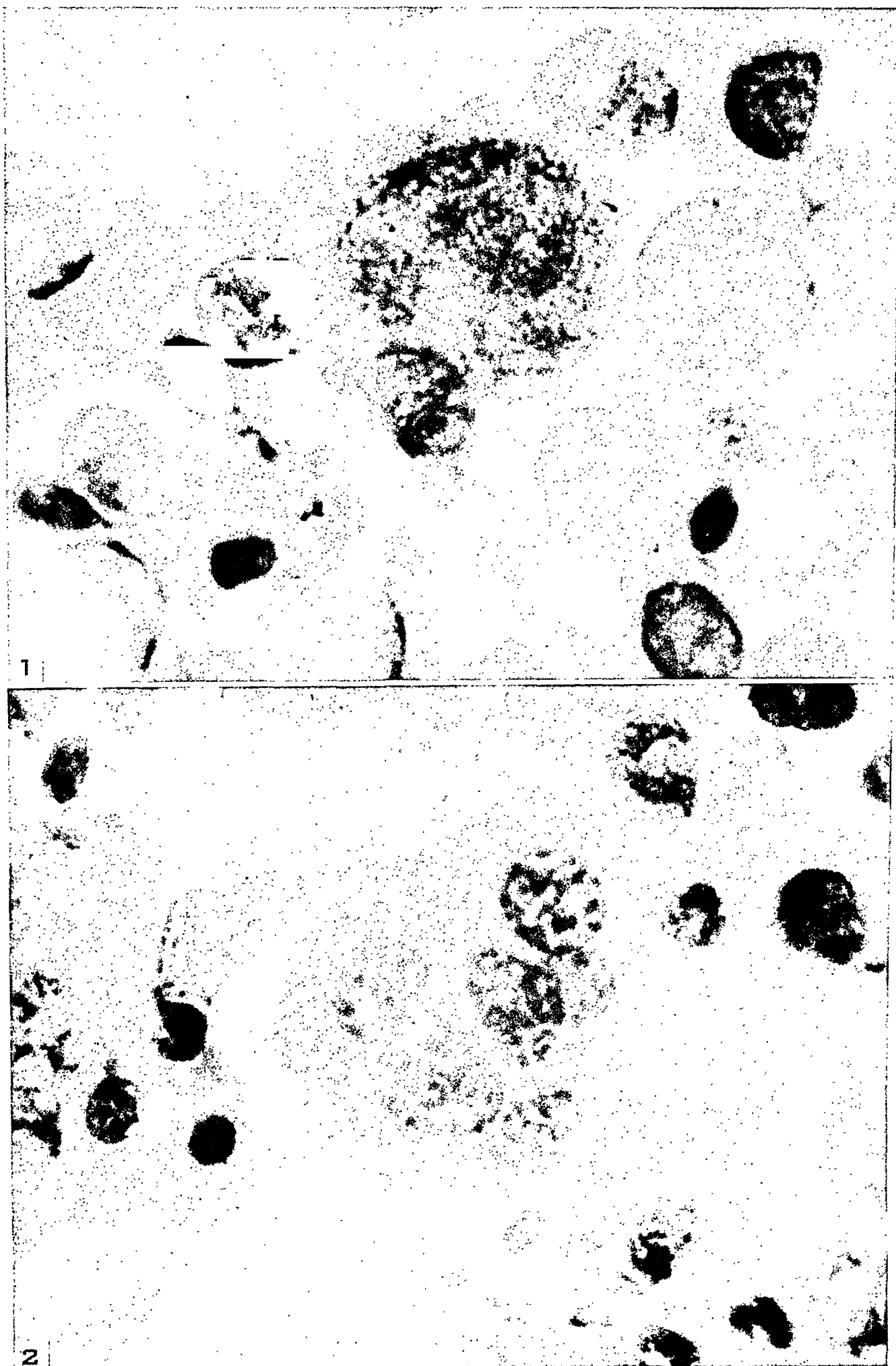
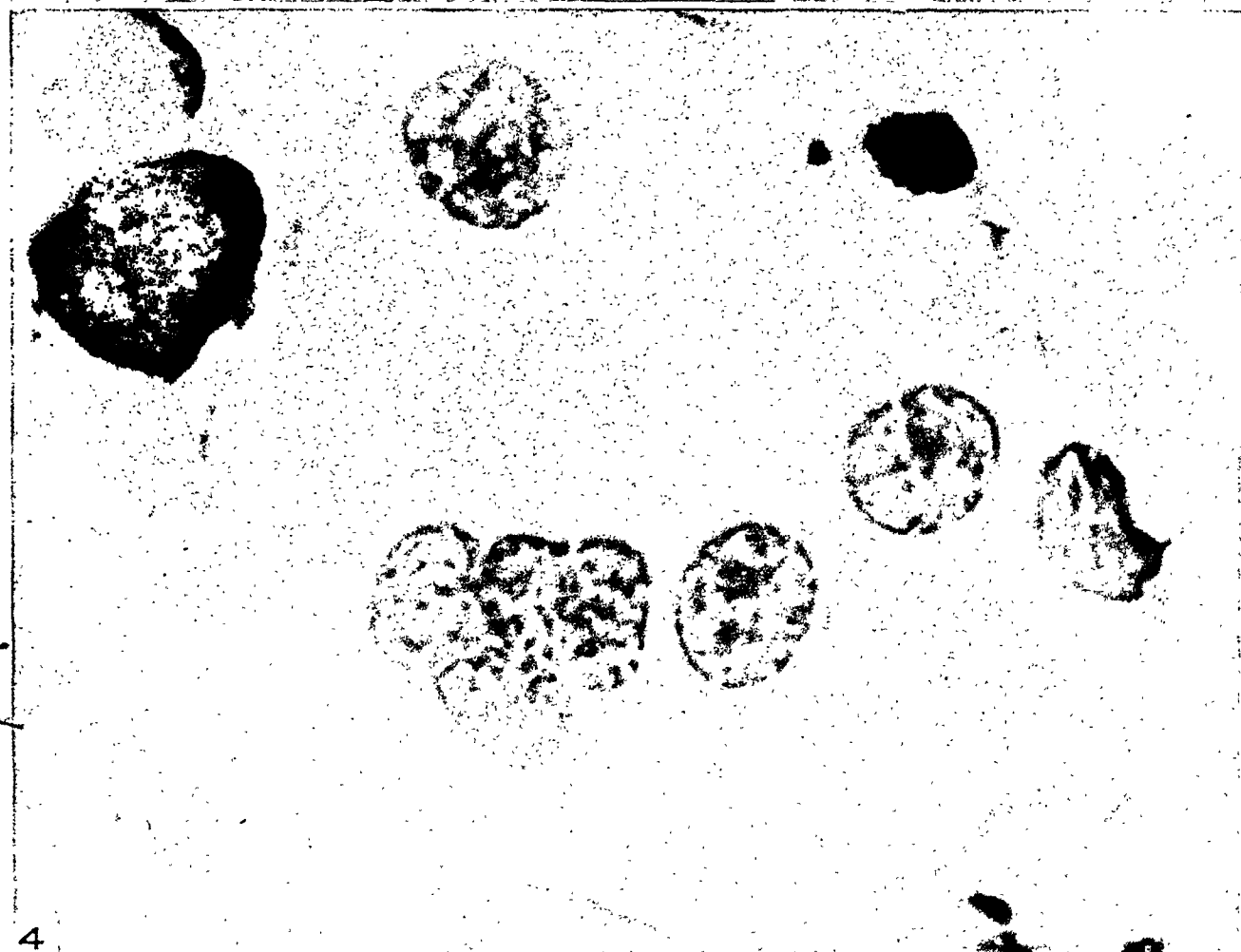
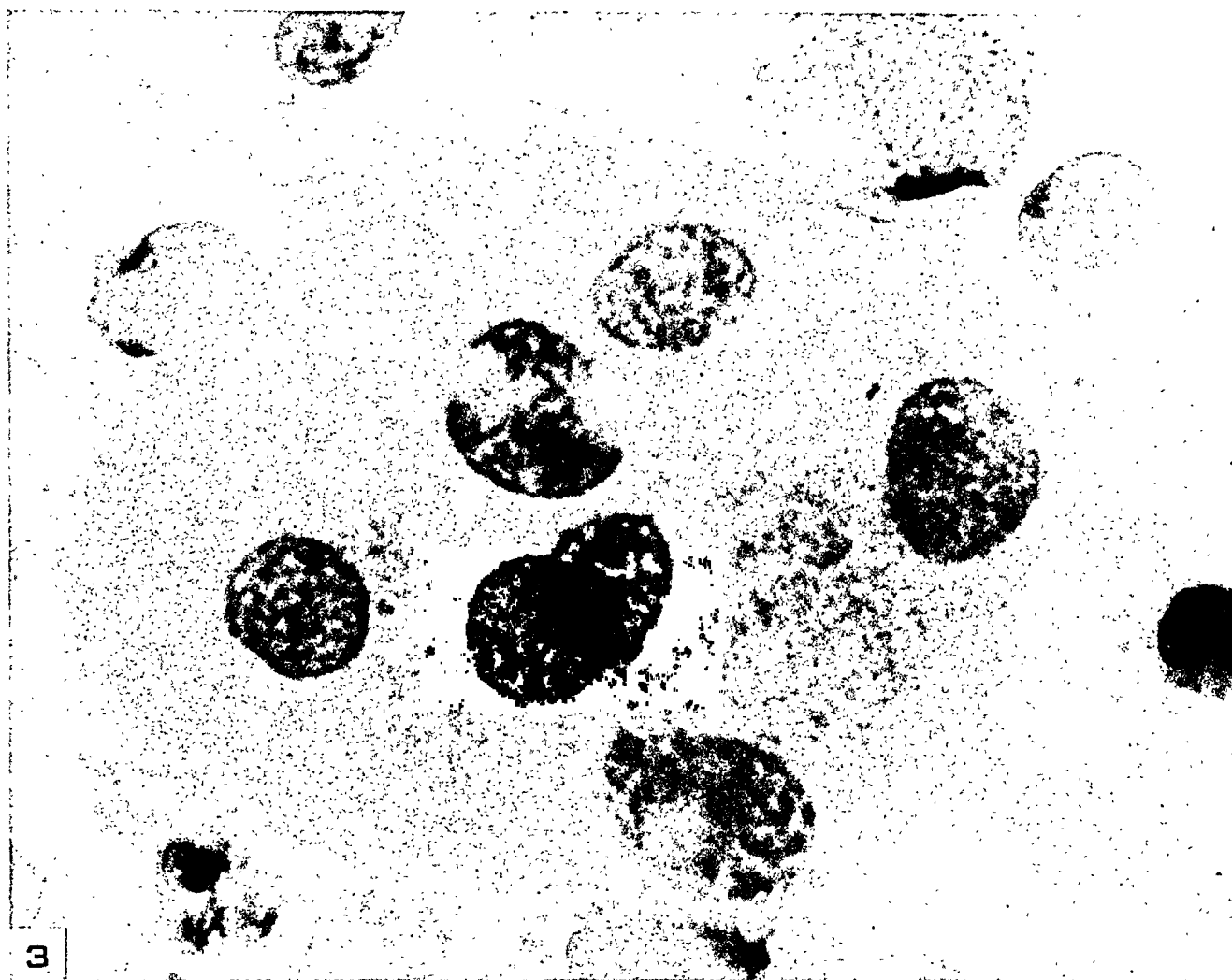


PLATE 34

FIG. 3. A "mature" polykaryocyte with 7 nuclei from case 2. The nuclear chromatin is slightly more dense and compact than that shown in Figure 2. The cytoplasm is free of basophilia and contains many granules. There is apparently some platelet formation.  $\times 1150$ .

FIG. 4. A "mature" polykaryocyte from case 3, showing grouping of 4 of the 8 nuclei.  $\times 1150$ .







# THE EFFECT OF NORMAL SALINE SOLUTION, RINGER'S LACTATE SOLUTION, AND DISTILLED WATER ON THE LUNGS OF DOGS AND RABBITS \*

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The original purpose of this study was to determine the effect of amniotic fluid, meconium, and vernix caseosa on pulmonary tissue. Meconium and vernix were suspended in normal saline solution because the latter had been found by Winternitz<sup>1</sup> to produce practically no polymorphonuclear exudate and only moderate capillary congestion and hemorrhage when introduced into the lungs of dogs in large quantities. In all dogs in which either meconium or vernix mixed with normal saline solution or amniotic fluid by itself was introduced into the lungs, we found a marked polymorphonuclear exudate in the alveoli and bronchi between 6 and 30 hours later. To exclude all possibility that this reaction was produced by the normal saline solution, we investigated the effect of this fluid on the lungs of dogs. We found an unanticipated polymorphonuclear exudate when saline solution alone was used, which was just as marked in its initial phase as when meconium and vernix were mixed with saline solution. These results led us to explore also the effect of distilled water and Ringer's lactate solution on the lungs of dogs and of saline solution on rabbits. The present report is concerned with the results of the latter experiments. The effects of amniotic fluid, meconium, and vernix on the lungs of dogs and rabbits will be reported separately.<sup>2</sup>

## METHODS

The normal saline solution used in these experiments was obtained from two sources. Ordinary tap water, which in our laboratory is alkaline, was distilled once in a metal still, using stainless steel and block tin as condensers. Following distillation, the water was tested for chlorides with silver nitrate and for available alkali with a methyl red, methylene blue indicator. Solutions containing chlorides or available alkali were rejected. Following the addition of 9 gm. of sodium chloride per l. of water, the solution was autoclaved without preservative being added. Fresh normal saline solution was made up every day. The above solution was used on all dogs numbered from 106 to 145 (Table I) and on all rabbits in Table II. The normal saline solution and distilled water used on dogs 145 through 152 were made by Abbott Laboratories and were pyrogen free and free of preservative. The Ringer's lactate solu-

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tion was made by diluting a 10 cc. ampule of lactate-Ringer's solution, made by Eli Lilly Co., twenty-five times with sterile distilled water (Abbott Laboratories).

Young, healthy, adult dogs and rabbits were used. The animals were given sulfadiazine and penicillin 1 hour or less before the introduction of the fluid into their lungs. Sulfadiazine was given subcutaneously in a 1 or 2 per cent solution in doses averaging  $\frac{1}{2}$  gr. per lb. of body weight. The dogs were given 100,000 units of penicillin in emulgen intramuscularly and the rabbits received half of this amount. If the experiment lasted 18 hours or longer, sulfadiazine and penicillin were given twice a day in the same amounts as originally administered.

Various anesthetics were used. Either nembutal intraperitoneally plus morphine sulfate intramuscularly or sodium pentothal intravenously was employed to induce surgical anesthesia. Both successfully abolished the cough reflexes of all animals. A few experiments were done using only a local anesthetic. After the skin of the neck had been infiltrated with 2 per cent novocaine, the trachea was exposed through a small incision and the solutions were introduced into the lung through a needle inserted between the tracheal rings.

All solutions were introduced either through a bronchoscope passed through the mouth into the trachea with its tip directed into the right or left main bronchus, or through a needle inserted into the trachea as described above. All solutions were sterile and in all experiments a sterile technic was employed, so that contamination of the lung was avoided wherever possible. The solutions were introduced into the lungs while the animals were kept in a supine position, with the head and shoulders elevated  $15^{\circ}$  to  $20^{\circ}$  above the rest of the body. They were maintained in this position for at least  $\frac{1}{2}$  hour after the solution had been introduced. The animals were allowed water but no food for the 18 hours preceding the experiment.

The amount of solution introduced into the lungs was carefully controlled, being set at 2 cc. per lb. of body weight. Except in rabbits, no difficulties were encountered in using this amount of fluid if about 5 minutes were allowed for it to run into the lung.

The animals were sacrificed from 15 minutes to 72 hours after the introduction of the fluid. Nembutal administered intravenously was used to stop the respirations. The abdomen was opened and the abdominal aorta cut prior to opening the chest and removing the lungs. The lungs were examined for gross changes and sections were taken from the more involved portions and placed in 10 per cent formalin. Histologic preparations were studied after staining with hematoxylin and eosin.

Cultures were made from the involved portions of the affected lobes of all animals. Using sterile precautions, a section of involved lung about 1 cc. in size was streaked across a blood agar plate and then ground in a mortar with about 4 cc. of sterile broth and carborundum. Two-tenths cc. of the ground mixture was transferred to each of the following: a blood agar plate, beef heart broth, and deep meat infusion broth. Blood cultures were obtained from the heart's blood at the time of sacrifice in some animals.

Rectal temperatures were obtained on many animals before and after the experiments and the animals were closely watched following the introduction of fluid into their lungs to note their reactions.

### RESULTS

The results of the experiments on dogs are given in Table I.

Within 1 hour after the introduction of normal saline solution into their lungs, 4 dogs (118 to 121) were sacrificed. Their lower lobes were heavy and distended, and mottled blue to purple. The cut surfaces were wet, and abundant, white, frothy material exuded from the bronchi, although sections taken from the involved portions of the lower lobes did not sink in the 10 per cent formalin used as a fixative. On histologic examination slight change only was seen. Congestion was minimal and there was only a rare polymorphonuclear leukocyte in the bronchi or alveoli. There was no pink-staining material in the alveoli or hemorrhage, as in dogs allowed to survive 6 hours or more.

Nineteen dogs were sacrificed 6 to 7 hours after the introduction of solution into their lungs. Studies were made on the possible effect of various anesthetics, solutions, and routes of administration of the solutions in this larger group. The gross appearance of their lungs, while presenting some individual differences, was in general fairly uniform. Portions of the lower and middle lobes in most instances appeared enlarged and dark red to purple. On section, fluid exuded from the cut surface and the bronchi contained white, frothy material. Only in a few instances was consolidation so extensive that portions of the lung sank in the fixative.

The histologic findings varied considerably in this group. In all of the lungs, polymorphonuclear leukocytes were found in the bronchi and alveoli. The degree of polymorphonuclear exudate in the bronchi and alveoli was graded in each instance on a scale ranging from 1 plus to 4 plus and the results are incorporated in Table I under the heading of "polymorphonuclear leukocytic exudate." A grade of 1 plus was assigned if the lungs had only an occasional polymorphonuclear leukocyte in the alveoli, as in dogs 137, 139, 149, and 150. If the alveoli and

TABLE I  
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, Isotonic Ringer's Lactate Solution, and Distilled Water by Bronchoscope or Intratracheally

TABLE I Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Cultures	Polymorpho-nuclear leukocytic exudate	MILLER, HAMILTON, WISE, AND	
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Ground lung			
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Ground lung			
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Lung smear	Blood-agar plate	Beef heart broth	Deep meat infusion broth
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Blood-agar plate	Blood-agar plate	Gram-negative rods	Staphylo-coccus*
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Negative	Negative	Negative	Staphylo-coccus*
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Negative	Negative	<i>H. influenzae canis</i>	Negative
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	<i>B. bronchiseptica</i>	<i>B. bronchiseptica</i>	Staphylo-coccus*	<i>B. bronchiseptica</i>
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Negative	Negative	Negative	Negative
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Negative	Negative	Negative	<i>B. bronchiseptica</i>
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Negative	Negative	Negative	Negative
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Negative	Negative	<i>B. bronchiseptica</i>	<i>B. bronchiseptica</i>
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Negative	Negative	<i>B. bronchiseptica</i>	<i>B. bronchiseptica</i>
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Negative	Negative	Negative	Negative
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Negative	Negative	Negative	Negative
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Negative	Negative	Negative	Negative
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Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Negative	Negative	Negative	Negative
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Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Negative	Negative	Negative	Negative
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Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Negative	Negative	Negative	Negative
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Negative	Negative	Negative	Negative
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Negative	Negative	Negative	Negative
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Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Negative	Negative	Negative	Negative
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Negative	Negative	Negative	Negative
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Negative	Negative	Negative	Negative
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Negative	Negative	Negative	Negative
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Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Negative	Negative	Negative	Negative
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Negative	Negative	Negative	Negative
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lactate Solution, and Distilled Water by Bronchoscope	Dog	Solution	Route administered	Duration of experiment	Anesthetic	Negative	Negative	Negative	Negative
Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, or Intratracheally Ringer's Lact									

136	Saline	Intratrach- eally	6 hrs.	Novocaine	<i>B. bronchisepti-</i> <i>ticus</i>	<i>B. bronchisepti-</i> <i>ticus</i>	<i>B. bronchisepti-</i> <i>ticus</i>	<i>B. bronchisepti-</i> <i>ticus</i>	++
137	Saline	Intratrach- eally	6 hrs.	Novocaine	Negative	Negative	Gram-positive rods	Gram-positive rods	+
138	Saline	Intratrach- eally	6 hrs.	Novocaine	<i>B. bronchisepti-</i> <i>ticus</i>	<i>B. bronchisepti-</i> <i>ticus</i>	<i>B. bronchisepti-</i> <i>ticus</i>	<i>B. bronchisepti-</i> <i>ticus</i>	++
139	Saline	Intratrach- eally	6 hrs.	Novocaine	<i>B. coli</i> and staphylo- coccus	<i>B. coli</i> and staphylo- coccus	<i>B. coli</i> and staphylo- coccus	<i>B. coli</i> and staphylo- coccus	+
140	Saline	Intratrach- eally	6 hrs.	Sodium pen- tothal, i.v.	Negative	Negative	Negative	Negative	++++
141	Saline	Intratrach- eally	6 hrs.	Sodium pen- tothal, i.v.	Negative	Negative	Negative	Negative	+++
145	Ringer's lactate	Intratrach- eally	6 hrs.	Sodium pen- tothal, i.v.					++
146	Ringer's lactate	Intratrach- eally	6 hrs.	Sodium pen- tothal, i.v.	<i>B. subtilis</i>	<i>B. subtilis</i>	<i>B. subtilis</i>	<i>B. subtilis</i>	+++
147	Distilled water	Intratrach- eally	6 hrs.	Sodium pen- tothal, i.v.	Negative	Negative	Gram-negative rods	Negative	++
148	Distilled water	Intratrach- eally	6 hrs.	Sodium pen- tothal, i.v.	Kurthia	Kurthia	Kurthia	Kurthia	++
151	Distilled water	Intratrach- eally	6 hrs.	Sodium pen- tothal, i.v.	<i>Pseudomonas fluorescens</i>	<i>Pseudomonas fluorescens</i>	<i>Pseudomonas fluorescens</i>	<i>Pseudomonas fluorescens</i>	++++
152	Distilled water	Intratrach- eally	6 hrs.	Sodium pen- tothal, i.v.	Negative	Negative	Negative	Negative	++

TABLE I (Cont'd.)

Dog	Solution	Route administered	Duration of experiment	Anesthetic	Cultures				Polymorpho-nuclear leukocytic exudate
					Lung smear	Ground lung			
						Blood-agar plate	Beef heart broth	Deep meat infusion broth	
149	Saline	Intratrach-eally	6 hrs.	Sodium pen-tothal, i.v.	Negative	Negative	Negative	Negative	+
150	Saline	Intratrach-eally	6 hrs.	Sodium pen-tothal, i.v.	Negative	Negative	Negative	Gram-negative rods	+
153	None		6 hrs.	Nembutal	Negative	Negative	Negative	Negative	o
154	None		6 hrs.	Nembutal	Negative	Negative	Negative	Negative	o
132	Saline	Intratrach-eally	17 hrs.	Nembutal	Negative	Negative	Negative	Staphylo-coccus	o
133	Saline	Intratrach-eally	18 hrs.	Nembutal	Negative	Negative	Negative	Negative	+
110	Saline	Bronchoscop-ically	24 hrs.	Nembutal	Negative	Negative	Negative	Gram-negative rods	++
109	Saline	Bronchoscop-ically	72 hrs.	Nembutal	Negative	Negative	Negative	Negative	+

\* Species not determined.

bronchi were packed with polymorphonuclear leukocytes, the condition was graded 4 plus, as was true of dogs 106, 122, 123, 134, 135, 140, and 151. If only patches of alveoli were filled with polymorphonuclear leukocytes, the grade was 2 plus or 3 plus depending on the extent of pulmonary involvement. Other changes were seen in histologic sections. Congestion of the capillaries sometimes was marked but appeared to have no relation to the number of polymorphonuclear leukocytes. Hemorrhage was observed in some sections in both bronchi and alveoli. Some dogs, particularly those with large numbers of polymorphonuclear leukocytes in the alveoli and bronchi, showed considerable homogeneous, pink-staining material, interpreted as serum protein, in the alveoli. There was no necrosis, but a minor degree of cellular infiltration was noted in the walls of the bronchi and bronchioles, and the columnar cells lining the bronchi occasionally were detached.

The gross appearance of the lungs was not always in close correlation with the microscopic findings. The lungs of dog 149 were distended and heavy in both lower lobes and had a meaty appearance on section, with abundant frothy material exuding from the bronchi, but received a grade of only 1 plus on microscopic examination. In contrast, dog 148 had minimal gross evidence of consolidation, but had a 2 plus polymorphonuclear exudate.

There appeared to be some relation between the degree of polymorphonuclear exudate in this group of 19 dogs and the method of anesthesia. The least cellular exudate was observed in the 4 dogs in which only local anesthesia with novocaine had been used (dogs 136 to 139), while the greatest amount of exudate was seen consistently in the 5 dogs receiving a combination of nembutal and morphine (dogs 106, 122, 123, 134, and 135). These dogs were frequently semi-stuporous and unable to get on their feet 6 hours after the introduction of the fluid into their lungs, while the dogs which were given only novocaine were up and alert as soon as they were removed from the table. Intermediate in effect was sodium pentothal, which was used on the remaining 10 dogs. Most of these animals were able to be up when they were removed from the operating table,  $\frac{1}{2}$  hour after the introduction of the solutions. The polymorphonuclear exudate also appeared to be intermediate in amount between those of the other two groups of dogs. Two of the dogs receiving sodium pentothal (140 and 151) had a 4 plus response, 2 others (149 and 150) had a 1 plus reaction, and the remaining 6 were graded as either 2 or 3 plus (141, 145, 146, 147, 148, and 152). To determine whether or not a general anesthetic had any direct effect on the polymorphonuclear response, 2 dogs (153 and 154) were injected with nem-



butal in an amount to produce surgical anesthesia and sacrificed 6 hours later. There were no changes in their lungs, either grossly or microscopically.

The effect of the anesthetic agent probably is only one of the variables in the polymorphonuclear response. The slight response in the dogs under local anesthesia might have been related to the very active cough reflex which was present during the introduction of saline solution into the lungs and which could have reduced the total amount of solution reaching the alveoli. The cough reflex was completely abolished in dogs under general anesthesia and these animals probably received the full amount of saline solution introduced.

The type of solution introduced into the lungs of the dogs apparently did not affect the degree of polymorphonuclear response, since tissue sections showed the same variations regardless of whether saline solution, Ringer's lactate solution, or distilled water was employed.

There appeared to be no relationship between the polymorphonuclear response and the results of the lung cultures. Dogs with negative cultures, *e.g.*, 140 and 141, had a marked polymorphonuclear exudation and dogs with positive cultures, *e.g.*, 139, had only a slight response. It is doubtful whether all of the organisms recovered were pathogenic, particularly such bacteria as *Kurthia* in dog 148, *Bacillus subtilis* in dog 146, and *B. bronchisepticus* in dogs 122, 123, 136, and 138. Furthermore, even if the above organisms were pathogenic, they must have been relatively few, since they were recovered only on incubation in broth cultures and rarely on the cultures made on blood-agar plates. The relative unimportance of such bacteria as might have been swept into the lung by the solutions is attested by failure to recognize bacteria, either singly or in clumps, in microscopic sections.

Four dogs were sacrificed from 17 to 72 hours after the saline solution was introduced into their lungs (dogs 109, 110, 132, and 133). The extent of the polymorphonuclear reaction in the alveoli was diminished in this group as compared to those examined at 6 hours. Three of the dogs (109, 132, and 133) had either no polymorphonuclear leukocytes in the alveoli and bronchi, or only a 1 plus reaction. However, all 3 showed marked capillary congestion and thickening of the alveolar walls. Dog 110 had a 2 plus polymorphonuclear reaction at 24 hours, associated with edema, round cell infiltration, and an apparent thickening of the interstitial framework.

None of the dogs receiving saline solution into their lungs appeared to have any toxemia following the experimental procedure. On 11 of the dogs listed in Table I rectal temperatures were taken immediately

before the experiments and again 6 hours afterwards. None of these had a higher temperature 6 hours after receiving saline solution than the upper range of body temperature ( $38^{\circ}$  to  $40^{\circ}\text{C.}$ ) found before the experiment was begun.

The results of gross and microscopic studies of the lungs of rabbits given normal saline solution intratracheally under nembutal anesthesia (Table II) were strikingly different from those obtained in the dogs sacrificed at comparable periods following the experimental procedure. At most, the lower lobes of the rabbits were darker and more mottled than the upper lobes, which were spared. Congestion appeared to be present, but the lower lobes were not distended as in the dogs. The cut surfaces were not wet and practically no white froth was seen in the bronchi. The microscopic studies revealed far less change than was seen in the dogs' lungs. Rarely were granulocytes seen in the bronchi or alveoli. The greatest polymorphonuclear reaction was seen in rabbits 7, 9, and 10, and in them no more than a 1 plus response was observed. In their lungs the polymorphonuclear leukocytes were found in collapsed alveoli and were associated with large mononuclear cells. Thickening of the interstitial framework was present. There rarely was any hemorrhage into the alveoli and then only a few red blood cells were seen outside the capillaries. The pink-staining material so commonly seen in the bronchi and alveoli of the dogs was almost completely absent from the lungs of the rabbits. The alveolar walls were thickened, the alveoli partly collapsed, and the capillaries filled with red blood cells and granulocytes in all of the rabbits examined. These changes were most noticeable 6 to 24 hours after the solution was introduced, but had not entirely disappeared at 48 and 72 hours.

Cultures made from the lungs of the rabbits were negative, with one exception. Rabbit 9 had staphylococci on the blood-agar plate streaked with lung tissue, but all remaining cultures from the lungs of this rabbit were sterile.

#### COMMENT

Considerable experimental evidence has been gathered to show that the lung reacts to irritating substances by an exudation of polymorphonuclear leukocytes into the alveoli and bronchi.<sup>1,3,4</sup> In this respect normal saline solution has not been considered particularly irritating, largely as the result of the investigations of Winternitz.<sup>1</sup> Winternitz, however, studied the effect of normal saline in the lungs of dogs at a time when the polymorphonuclear reaction would not be expected to be maximum, according to the results obtained in the present study. He sacrificed dogs (1) within an hour of the injection of saline solution, (2) after

TABLE II  
*Bacteriologic and Pathologic Findings on Rabbits Receiving Normal Saline Solution Intratracheally*

Rabbit	Solution	Duration of experiment	Anesthetic	Cultures				Polymorpho-nuclear leukocytic exudate
				Lung smear	Ground lung			
				Blood-agar plate	Blood-agar plate	Beef heart broth	Deep meat infusion broth	
12	Saline	3 hrs.	Nembutal	Negative	Negative			o
11	Saline	6 hrs.	Nembutal	Negative	Negative			o
9	Saline	6 hrs.	Nembutal	Staphylococcus	Negative	Negative	Negative	+
5	Saline	7 hrs.	Nembutal	Negative				o
7	Saline	20 hrs.	Nembutal	Negative	Negative			+
8	Saline	20 hrs.	Nembutal	Negative	Negative	Negative	Negative	o
10	Saline	24 hrs.	Nembutal	Negative	Negative	Negative	Negative	+
13	Saline	48 hrs.	Nembutal	Negative	Negative	Negative	Negative	o
14	Saline	72 hrs.	Nembutal	Negative	Negative	Negative	Negative	o

recovery from ether, and (3) 18 hours or more after the introduction of saline solution into the lungs, thus missing the more marked polymorphonuclear reaction which comes about 6 hours after introduction of the fluid. Our results parallel his in that we found a negligible reaction in the first hour and a subsiding response from 18 to 72 hours after the saline solution was given. The reaction continues to subside, according to Winternitz' investigations in dogs, as he found only minimal changes on the fourth day and entirely normal lungs on the tenth day.

The present experiments were designed to eliminate, so far as possible, factors which in themselves might lead to an exudative response in the alveoli, such as infection and aspiration. Bacteria were recovered from some of the lungs of dogs, but almost wholly in the broth cultures of ground lung in which one organism would be sufficient to give a positive culture. Bacteria were seldom recovered on solid media, thereby providing a general index of the infrequency with which organisms reached the alveoli. Furthermore, there were 2 dogs, 140 and 141, in which all of the cultures were negative and yet the exudate of polymorphonuclear leukocytes was 4 and 3 plus, respectively. It is also believed that aspiration of saliva and mucus from the nose and throat could not have been a responsible factor in the production of the polymorphonuclear response in the lungs of the dogs, since positive lung cultures and a more diversified flora in individual dogs would undoubtedly have been found more frequently if such had been the case.

The pathologic picture seen in the lungs of dogs in the present study resembles that described under a variety of circumstances. According to Robertson,<sup>4</sup> the exudation of blood cells, principally polymorphonuclear leukocytes, along with large numbers of red blood cells and plasma, as seen in the dogs sacrificed at 6 hours in the experiments reported here, characterizes the initial response to highly irritating living and non-living materials. It is the same response as is seen in the early stages of pneumococcal pneumonia, "terminal" pneumonia, pneumonia seen in pulmonary passive congestion, and in shock<sup>5</sup> and postoperative pneumonitis.<sup>6</sup> The results of the present experiments on dogs indicate that the polymorphonuclear response in the lung is readily elicited in certain species even when relatively bland substances are introduced. The response of the lungs to solutions which approximate the concentration of electrolytes in the plasma indicates that some modification of current views may be necessary regarding the degree of irritation needed to produce polymorphonuclear exudation.

The minimal changes in the lungs of rabbits following the introduction of saline solution are in contrast to the marked polymorphonuclear

reaction seen in dogs. No explanation for the wide differences observed in the two species can be given. Saline solution is not the only substance, however, that has produced a different response in the lungs of these two species. The injection of virulent pneumococci intratracheally in rabbits in order to produce lobar pneumonia has resulted in a succession of almost complete failures,<sup>7</sup> whereas the injection in dogs has met with a high degree of success.<sup>8</sup> The relative difficulty encountered in producing lobar pneumonia in rabbits has never been satisfactorily explained. The lung of the dog appears to be more nearly like that of man in its response to virulent pneumococci. This similarity favors the hypothesis that the reaction of the human lung to saline solution, water, and Ringer's lactate solution would resemble that of the dog rather than that of the rabbit.

### CONCLUSIONS

Sterile normal saline solution, Ringer's lactate solution, and distilled water produced a significant polymorphonuclear exudate in the alveoli and bronchi of dogs within 6 hours of their introduction into the lungs. The polymorphonuclear response was subsiding within 18 to 24 hours after the solutions had been introduced.

The degree of polymorphonuclear exudate appeared to be greater in dogs under deep and prolonged anesthesia than in those under light anesthesia. The manner of administration and the type of solution did not appear to affect the degree of polymorphonuclear exudate in the lungs of dogs.

Sterile normal saline solution introduced into the lungs of rabbits resulted in little or no polymorphonuclear exudate.

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## MYOCARDITIS IN VITAMIN E-DEFICIENT RABBITS \*

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Vitamin E deficiency in the rabbit manifests itself in muscular weakness which, if the animal survives long enough, terminates in extensive paralysis. Pathologically, the voluntary muscles show what has long been recognized in man as Zenker's degeneration. Most of the earlier publications on this "nutritional muscular dystrophy" either make no reference to myocardial studies or contain the statement that the heart is unaffected.<sup>1-3</sup> On the other hand, some observations have been published indicating that myocardial damage also occurs. Madsen,<sup>4</sup> in 1936, described and illustrated degenerative and infiltrative lesions in the myocardium of dystrophic rabbits, but drew no conclusions. Houchin and Smith,<sup>5</sup> the first to claim a causal relationship between tocopherol deficiency and myocardial damage, demonstrated that slices of heart muscle from dystrophic rabbits, like slices of skeletal muscle, exhibit an increased oxygen consumption. They also showed in the intact deficient rabbit an increased sensitivity to pitressin and a greater resistance to ouabain and digoxin. More recently, Gatz and Houchin<sup>6,7</sup> have reported electrocardiographic and histologic changes regarded by them as evidence of myocardial degeneration in E-deficient rabbits.

It is the purpose of this paper to report additional observations, both electrocardiographic and morphologic, on myocardial abnormalities in rabbits receiving E-deficient diets.

### METHODS

Young male and female white rabbits weighing between 600 and 1300 gm., housed in individual cages on wire grids, were offered the following simplified E-deficient diets *ad libitum*. The two diets differ primarily in fat content. Animals gaining weight consumed 35 to 45 gm. of diet daily.

	Diet 1	Diet 2
Vitamin-free casein . . . . .	15 per cent	15 per cent
Lard . . . . .	20 per cent	10 per cent
Sucrose . . . . .	59 per cent	70 per cent
Salt mixture † . . . . .	6 per cent	5 per cent

Both diets were supplemented with equal quantities of vitamins.‡ Each

\* Received for publication, March 11, 1948.

† Formula of "salts 4" (Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B. Choline in the nutrition of chicks. *J. Biol. Chem.*, 1941, 138, 459-466).

‡ Vitamins were added in the following quantities per 100 gm. of diet; Thiamine, 1 mg.; pyridoxine, 1.5 mg.; riboflavin, 2 mg.; calcium pantothenate, 3 mg.; nicotinic acid, 30 mg.; choline chloride, 300 mg.; vitamin A (afaxin), 1000 units; and vitamin D (driisdol), 100 units. All vitamins were supplied through the courtesy of Winthrop-Stearns, Inc., New York 13, N.Y.

animal also received 0.5 cc. of a refined corn oil (mazola) three times a week. For the control animals, 15 mg. of alpha-tocopherol were dissolved in each 0.5 cc. of corn oil. Roughage was supplied from time to time in the form of filter paper.

The electrocardiograms were taken with a portable clinical machine (cardiette) in the three conventional leads only. The animals were unanesthetized but blindfolded.

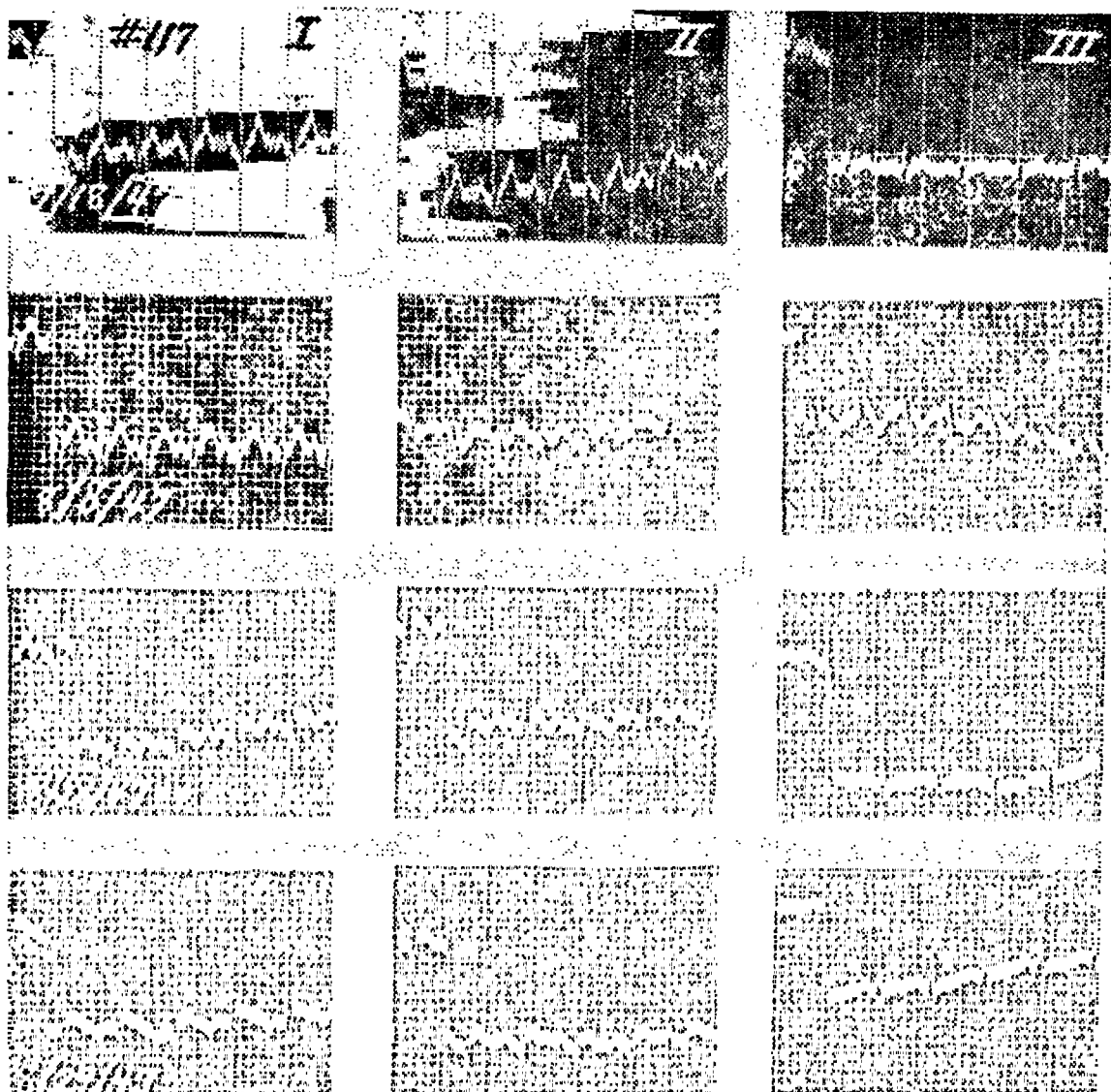
## RESULTS

Of 19 rabbits receiving the deficient diets, 17 survived long enough to develop clinical signs of muscular dystrophy; they comprise the material for this report. Twelve of these animals received diet 2; and 5, diet 1. All of the former group gained weight for about 4 weeks, then sickened, and were either sacrificed or died, usually in the 7th week. The 5 rabbits on the higher fat diet failed to gain weight. They developed signs of dystrophy and died, on the average, 1 week earlier than those on the lower fat diet.

There were 6 control rabbits, 3 on each diet. None developed muscular dystrophy. Four of them continued to gain weight until they were sacrificed 6 to 8 weeks later. The other 2, during the terminal weeks, showed a significant loss of weight, presumably the result of severe coccidial cirrhosis as demonstrated at necropsy.

Electrocardiograms were taken only on rabbits receiving diet 2. Seven of the deficient animals had two or more tracings. In each case a control tracing was taken before the animal was placed on the diet and another was taken immediately prior to sacrifice. The later tracings of 5 of these animals showed changes that are interpreted as definite abnormalities. These include elevation of the S-T segment and inversion of T waves in lead II (Text-Figs. 1 and 2). These changes did not develop until the latter part of the period of observation, when muscular dystrophy was already severe. They did not occur in any of the control animals receiving the same diet supplemented with alpha-tocopherol; nor were they ever observed in a series of 23 "normal" rabbits.<sup>8</sup>

All 17 rabbits on the deficient diets showed the typical gross and microscopic changes in their skeletal muscle described by Goettsch and Pappenheimer<sup>1</sup> in nutritional muscular dystrophy. In addition, 15 of them showed foci of necrosis accompanied by an inflammatory reaction in the myocardium (Figs. 1 to 3). The sequence of morphologic events in the cardiac muscle resembled that in the voluntary muscle and appeared to be as follows: A coagulative necrosis of sarcoplasm with loss of striations; pyknosis and later karyorrhexis of muscle nuclei; and an



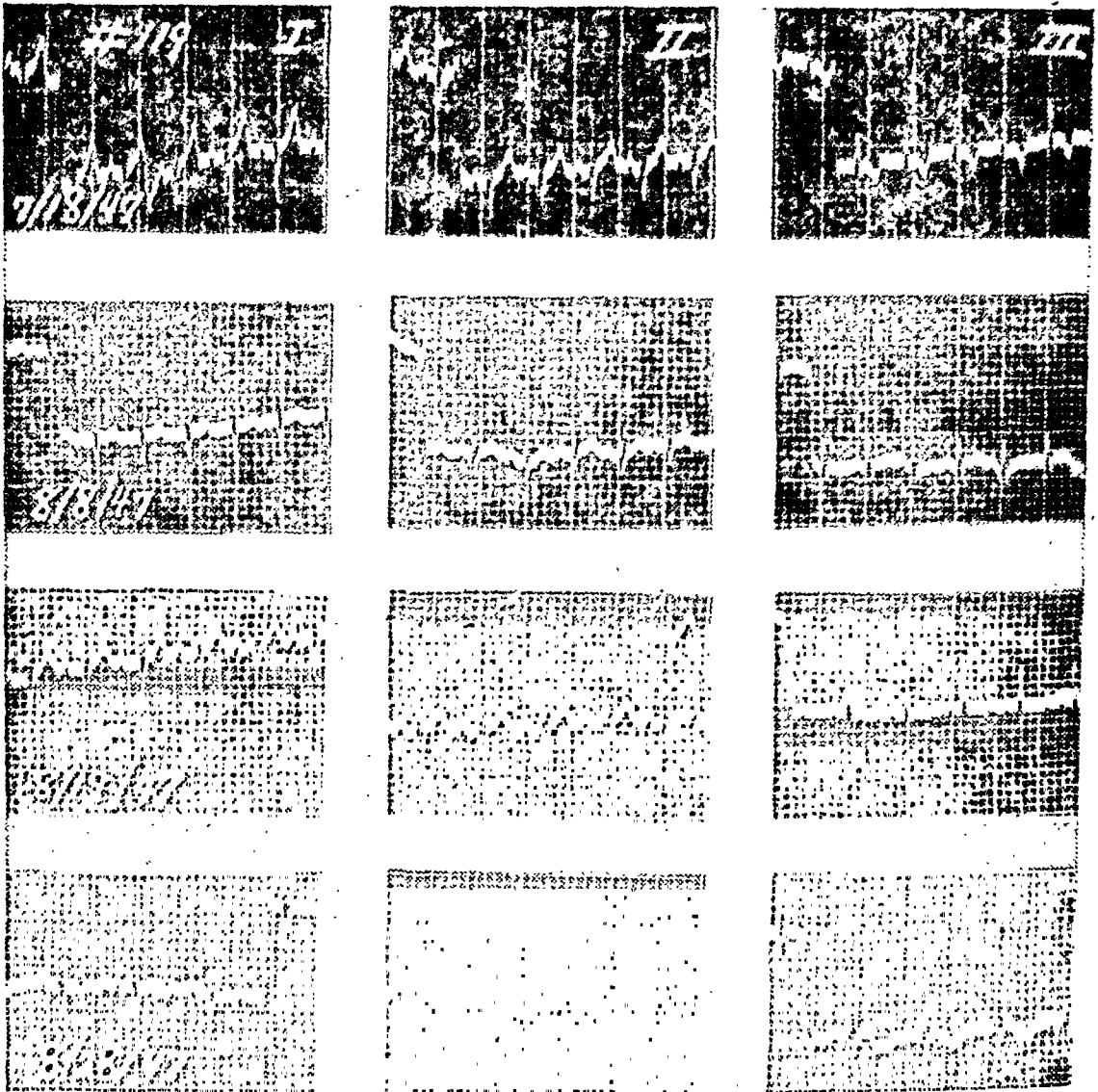
Text-Figure 1. Serial, conventional lead, electrocardiograms in vitamin E-deficient rabbit 117. Control tracing was taken on July 18, 1947. Reversal of T 3 on August 8 is a normal variation. Elevation of S-T 1 and 2 on August 28 and inverted T 1 and 2 on August 29 are abnormal findings. The animal was sacrificed the same day and necropsy revealed grossly visible areas of necrosis in the wall of the left ventricle.

inflammatory reaction characterized by edema, small hemorrhages, and the infiltration of monocytes and polymorphonuclear neutrophils in varying proportions.\* In animals in which lesions of the skeletal muscle showed calcification, there was usually calcification of necrotic cardiac muscle as well. There was no clear evidence of regeneration of cardiac muscle. Myocarditis was consistently more extensive in the 5 animals receiving the higher fat diet.

The most frequently involved sites were the posterior walls of the

\* In several instances sections were examined with the Gram stain. Even in animals that had lain dead overnight at room temperature, no organisms were found in the heart lesions.





Text-Figure 2. Serial electrocardiograms in vitamin E-deficient rabbit 119. Control tracing was taken on July 18, 1947. Tracing of August 8 shows low voltage but is not otherwise remarkable. On August 19, P-R was prolonged to 0.09 seconds in lead I (upper limit of normal), but this increase is partly explained by cardiac slowing. On August 28, inversion of T 1, 2, and 3 can be seen despite marked somatic tremor. This is an abnormal finding. The animal was found dead the following morning. Necropsy showed grossly evident lesions in both ventricles, ascites, hydrothorax, and passive congestion of viscera.

ventricles and the papillary muscles. In several animals the auricular muscle also was involved. When severe, the lesions were readily visible grossly as rather well circumscribed, gray areas. In one animal (119), the changes were more diffuse and were accompanied by the usual signs of congestive heart failure.

From the control animals the same number of sections were cut as from the deficient group. In the wall of the left ventricle of one rabbit there was a solitary lesion characterized by basophilia of the sarcoplasm

with loss of striations but without inflammatory reaction. In the other 5 animals no myocardial lesions were observed.

In addition to these changes in voluntary and cardiac muscle, 3 of the 5 animals receiving deficient diet no. 1 showed foci of necrosis and polymorphonuclear infiltration in the smooth muscle of the stomach and in scattered arterioles, particularly of the lung. These findings were most severe, even grossly obvious, in the gastric fundus. Although never present in the controls, these lesions were not encountered frequently enough to warrant relating them to tocopherol deficiency.

### DISCUSSION

That the myocarditis observed in these rabbits was the direct result of the tocopherol deficiency is evidenced primarily by the frequency of its occurrence in the deficient group (88 per cent) compared with its absence in the control group. On the other hand, other investigators have observed no heart lesions in severely dystrophic rabbits. The possibility cannot be excluded that these lesions are coincidental findings facilitated rather than caused by the vitamin deficiency. Because myocarditis of unknown etiology has been observed in "normal" rabbits, it should be emphasized that the animals in this series were young and that the lesions were all acute.

Although the lesions in the heart were neither so extensive nor so diffuse as those in the skeletal muscle, the differences were essentially quantitative. It has been shown that in rats on a low tocopherol intake the heart contains proportionately twice as much of the vitamin as does the skeletal muscle.<sup>9</sup> A comparable distribution in the rabbit might explain not only the difference in the extent of the lesions but also the delay in the appearance of electrocardiographic changes until the muscular dystrophy was well advanced. Return of these changes to normal by the addition of tocopherol was not attempted because at the stage in which they appeared the animals were considered beyond recovery.

The physiologic rôle of vitamin E remains unknown. Although its deficiency in various animals produces strikingly similar lesions in skeletal muscle, there remain peculiar species specificities. These include encephalomalacia in the chick,<sup>10</sup> irreparable degeneration of germinal epithelium in the rat,<sup>11</sup> and necrosis of smooth muscle in the gizzard of the turkey.<sup>12</sup> Mason and Emmel<sup>13</sup> observed myocardial lesions of a chronic nature in rats maintained for over 1 year on an E-deficient diet. The only other species in which myocardial damage has been claimed in this deficiency is the cow.<sup>14</sup> Further generalizations are not warranted.

## SUMMARY

Among 17 rabbits that developed severe muscular dystrophy on vitamin E-deficient diets, 15 (88 per cent) showed foci of acute myocarditis at necropsy. In some cases there were abnormal electrocardiographic changes as well. Animals receiving the same diets supplemented with alpha-tocopherol failed to show these changes.

The sequence of pathologic events in cardiac muscle resembled the changes in voluntary muscle, although the lesions in the former were less diffuse.

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## DESCRIPTION OF PLATE

## PLATE 35

- FIG. 1. Rabbit A3. Section of the wall of the right ventricle and pulmonic cusp. This animal became severely dystrophic and was sacrificed after  $4\frac{1}{2}$  weeks on diet 1. There had been no weight loss.  $\times 100$ .
- FIG. 2. Rabbit A2. A rather typical lesion in the ventricular wall, showing necrosis and fragmentation of sarcoplasm with complete loss of normal structure and infiltration of inflammatory cells.  $\times 800$ .
- FIG. 3. Rabbit 129. Fragmentation of a single myocardial fiber and polymorphonuclear infiltration.  $\times 800$ .





# FACTORS INFLUENCING COLLAGEN CONTENT IN EXPERIMENTAL CIRRHOSIS \*

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In a previous study,<sup>1</sup> the increases in collagen which occur in experimental cirrhosis due to carbon tetrachloride and *p*-dimethylaminoazobenzene were studied by chemical means. It was shown, also, that partial or complete disappearance of collagen occurs after the administration of these hepatotoxic substances is stopped. The present observations concern the rate at which collagen is deposited and resorbed in experimental cirrhosis, as well as factors which influence these changes.

## METHODS

A total of 252 male albino rats, weighing 180 to 220 gm. each, were employed. Hepatic cirrhosis was produced in 234. The rats, in groups of approximately 40, were placed in an air-tight, glass-topped chamber. Carbon tetrachloride vapor was introduced into the chamber until the animals became semi-comatose. They were maintained in this state for several minutes. The concentration of the vapor was then raised until the animals became unconscious. The glass top was then quickly removed and the interior of the chamber was ventilated by vigorous fanning. The rats regained consciousness rapidly. This procedure was repeated three times during the course of 20 minutes. Exposure to carbon tetrachloride was repeated every other day for a period of 35 days. Groups of animals were sacrificed at 5-day intervals, and collagen determinations were performed on 2 to 3 gm. samples of their livers in order to determine the rate of collagen deposition. On the 36th day, the remaining rats, selected at random, were divided into 7 groups. One group was left on a normal diet; from these, animals were periodically sacrificed to ascertain the rate of collagen resorption. Five groups of 20 rats each were placed on special diets. Ligation of the portal vein was performed on the last group with cirrhosis according to the method of Whitaker.<sup>2</sup> Six normal control rats were sacrificed to determine the normal value of hepatic collagen, and ligations of the portal vein were performed on 12 additional normal rats.

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The method of Lowry, Gilligan, and Katersky<sup>3</sup> was modified as follows, in order to determine quantitatively the collagen content of liver: Pound and grind the sample thoroughly. Transfer to a round-bottomed, 50 cc. pyrex centrifuge tube with 40 cc. of distilled water. Centrifuge and discard the supernatant liquid. Add 40 cc. of distilled water and allow to stand for 5 minutes. Centrifuge and discard the supernatant liquid. Add 40 cc. of 0.1 normal sodium hydroxide, stir, and allow to stand for 5 minutes. Centrifuge and remove the supernatant liquid. Add 40 cc. of 0.1 normal sodium hydroxide, stir, and let stand overnight at room temperature. Stir, centrifuge, and remove the supernatant liquid. Add 40 cc. of 0.1 normal sodium hydroxide and let stand 1 hour with frequent stirring. Centrifuge and remove the supernatant liquid. Add 40 cc. of distilled water and 1 drop of 0.1 per cent phenol red indicator. Adjust color to faint pink with 0.1 normal hydrochloric acid, allowing time for diffusion of alkali from the tissue fragments. Centrifuge, and discard the supernatant liquid. Add 40 cc. of a mixture of 3 parts of 95 per cent alcohol and 1 part absolute ether. Let stand for 20 minutes with occasional stirring; centrifuge and remove the supernatant liquid. Add 40 cc. of absolute ether, mix, and let stand for 10 minutes. Centrifuge and remove the supernatant liquid. Dry to constant weight in an oven at 100° C. (2 to 4 hours), and add 40 cc. of distilled water. Stopper the tubes with nonabsorbent cotton and autoclave for 4 hours, at 50 lbs. pressure. Cool, centrifuge, discard the supernatant liquid, and dry to constant weight. The weight before autoclaving minus the weight after autoclaving represents the weight of collagen in the sample, since the collagen is converted by the autoclaving into soluble gelatin.

All animals were autopsied and sections of the livers were stained with hematoxylin and eosin, Laidlaw's reticulum stain counterstained with van Gieson's mixture, and Masson's trichrome stain.

## RESULTS

The increases in collagen observed with progressive cirrhosis are shown in Table I and in Text-Figure 1. Elevated values were noted in both the percentage of collagen and in the total hepatic collagen. The deposition of collagen shown in Text-Figure 1 follows a curve of exponential type. The early lag phase most probably represents the early proliferation of fibroblasts, which progresses in a 1, 2, 4, 8, 16-manner. Collagen formation accordingly occurs at a relatively greater rate as geometric fibroblastic multiplication proceeds.

The lower normal value for collagen content, *i.e.*, 0.16 per cent (Table I), as compared to 0.23 per cent found in a previous study, may be

accounted for by the modified procedure which was employed in the present experiments. The preliminary extraction with water, however, is advisable with liver in order to avoid the markedly viscous solution which results when dilute alkali is employed for the first extraction.

An explanation for the irregularities in the reversal phase shown in Text-Figure 1 may be found in the fact that fewer animals (25 rats) were employed in this phase as compared to the 57 which were used to determine the rate of collagen deposition. The incompleteness of the

TABLE I  
*Collagen Content in Hepatic Cirrhosis Due to Carbon Tetrachloride*

Days	Rat no.	Weight of rat	Weight of liver	Collagen, wet weight	Total hepatic collagen	Average collagen, wet weight for the group
		gm.	gm.	per cent	mg.	per cent
Normal	1	182.0	10.7	0.24	25.7	0.16
	2	188.8	10.6	0.14	14.8	
	3	204.0	12.5	0.12	15.0	
	4	207.1	12.1	0.12	14.5	
	5	224.5	13.5	0.21	28.4	
	6	227.3	13.4	0.10	13.4	
5	7	182.1	9.2	0.21	19.3	0.18
	8	188.1	10.5	0.19	19.9	
	9	192.3	10.4	0.18	18.7	
	10	203.1	10.1	0.15	15.2	
	11	212.8	11.3	0.16	18.1	
10	12	145.5	10.2	0.19	19.6	0.22
	13	173.1	13.2	0.11	14.5	
	14	190.7	11.7	0.18	21.1	
	15	205.4	11.2	0.20	22.4	
	16	207.0	10.6	0.29	30.7	
	17	216.3	15.1	0.33	49.8	
15	18	174.9	9.4	0.27	25.4	0.28
	19	180.4	12.3	0.34	41.8	
	20	183.4	12.5	0.36	44.0	
	21	190.2	10.4	0.24	24.9	
	22	224.5	18.0	0.25	45.0	
	23	242.9	15.5	0.21	32.6	
20	24	116.0	5.6	0.27	15.1	0.31
	25	135.6	8.5	0.48	40.8	
	26	137.5	11.1	0.25	27.8	
	27	157.0	10.0	0.35	35.5	
	28	199.2	14.2	0.40	56.8	
	29	201.7	12.6	0.25	31.5	
	30	220.1	14.4	0.23	33.1	
	31	270.7	13.1	0.22	28.8	
25	32	122.4	6.7	0.49	31.9	0.37
	33	144.6	12.0	0.50	60.0	
	34	151.9	13.9	0.28	38.9	
	35	160.5	10.0	0.28	28.0	
	36	164.9	17.5	0.46	80.5	
	37	176.1	12.5	0.24	30.0	
	38	189.6	11.4	0.37	42.2	
	39	199.7	11.4	0.43	49.0	
	40	207.9	12.2	0.26	31.7	



## MORRIONE

TABLE I (Con't.)

Days	Rat no.	Weight of rat	Weight of liver	Collagen, wet weight	Total hepatic collagen	Average collagen, wet weight for the group
30	41	gm. 103.4	gm. 6.6	per cent 0.63	mg. 41.6	per cent 0.52
	42	130.0	12.9	0.45	58.1	
	43	142.3	9.6	0.49	47.0	
35	44	108.4	6.0	0.40	24.0	0.54
	45	116.4	7.4	0.78	57.7	
	46	131.7	9.5	0.76	72.2	
	47	131.8	5.7	0.45	25.7	
	48	150.6	13.2	0.42	45.4	
	49	153.8	10.0	0.54	54.0	
	50	155.0	11.4	0.36	41.0	
	51	158.7	9.9	0.73	72.3	
	52	163.0	10.9	0.45	49.1	
	53	166.3	13.0	0.40	43.6	
	54	171.2	12.6	0.76	98.8	
	55	179.2	13.7	0.64	80.6	
	56	186.5	13.5	0.50	68.5	
	57	215.8		0.36	48.6	

## Reversal of cirrhosis on normal diet\*

3†	58	87.3	5.2	0.62	32.2	0.51
	59	116.1	5.7	0.83	47.3	
	60	132.1	11.0	0.35	38.5	
	61	132.6	9.8	0.30	29.4	
	62	156.2	12.1	0.70	84.7	
	63	159.7	8.9	0.31	27.6	0.40
	64	193.6	12.3	0.44	54.1	
7†	65	118.1	7.5	0.29	21.8	
	66	133.0	7.9	0.57	45.0	
	67	148.4	8.8	0.35	30.8	
	68	153.1	8.1	0.48	38.9	0.38
	69	244.4	14.4	0.30	43.2	
21†	70	123.3	7.0	0.31	21.7	
	71	195.3	10.7	0.45	48.2	
	72	211.3	10.0	0.39	39.0	0.24
	73	233.1	11.1	0.35	38.9	
28†	74	176.3	8.0	0.28	22.4	
	75	219.1	9.4	0.21	19.7	
	76	240.2	10.3	0.23	23.7	0.36
31†	77	185.8	7.7	0.28	21.6	
	78	241.6	9.8	0.44	43.1	
38†	79	153.5	7.6	0.33	25.1	
	80	176.2	9.3	0.20	27.0	
	81	181.9	8.4	0.39	32.8	0.32
	82	227.5	10.8	0.28	30.2	

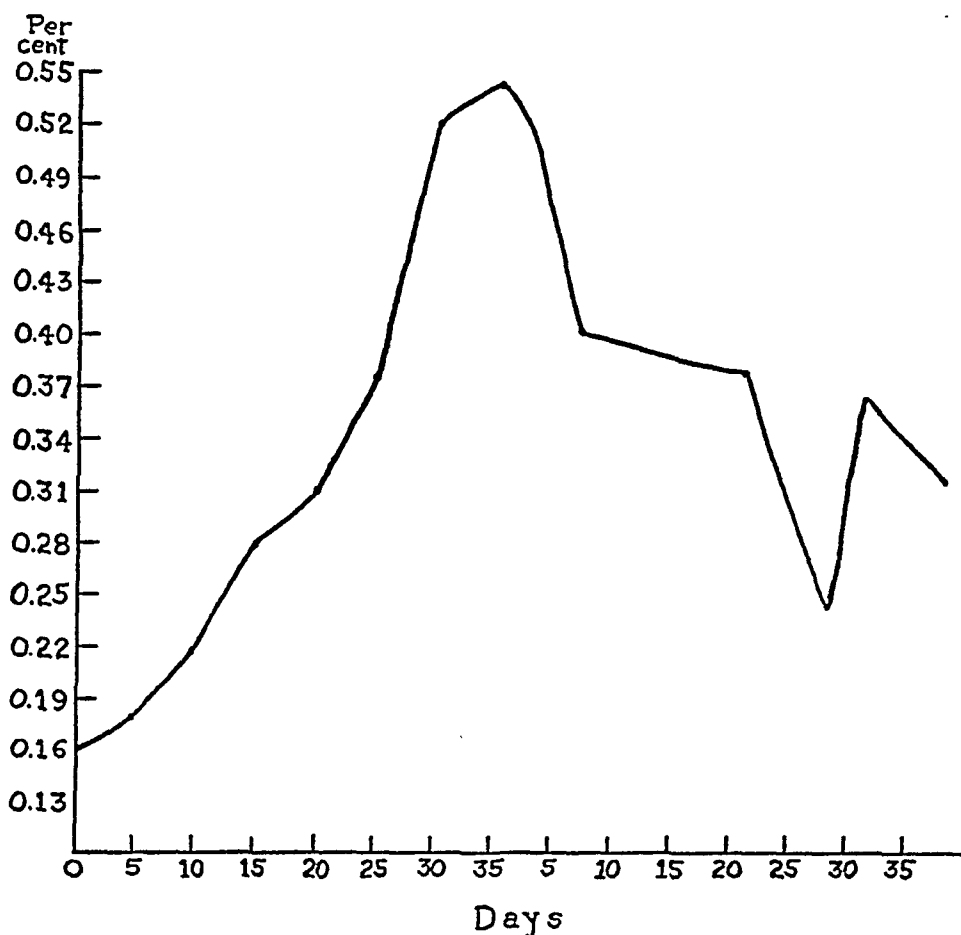
\* Dog chow pellets.

† Days after last exposure to carbon tetrachloride.

reversal which took place is shown by the 0.32 per cent collagen content level at 38 days, as well as by microscopic evidence of persisting cirrhosis in these animals.

*Reversal of Cirrhosis on Special Diets*

One hundred rats, on carbon tetrachloride for 35 days, were placed on special diets the day after the carbon tetrachloride was stopped. Twenty animals were employed in each of 5 groups. The diets used are shown in Table II.



Text-Figure 1. Graphic representation of data presented in Table I. Alterations in collagen content in cirrhosis due to carbon tetrachloride. The drug was discontinued on the 35th day.

All diets were fed to the animals with cirrhosis for a period of 35 days. Decreases in hepatic collagen, determined chemically and confirmed by microscopic study, were observed with all five diets. The greatest decrease occurred with diet B, a low protein diet supplemented with methionine, choline, and cystine. The arithmetic mean of the hepatic collagen content after 35 days on diet B was 0.28 per cent, as compared to 0.47 per cent with diet D, a low protein, high fat diet. On diets A, C, and E, the average collagen content was 0.36, 0.34, and 0.36 per cent, respectively, after 35 days of reversal on these diets. These latter values are not significantly different from the level of 0.32 per cent which was

observed after 38 days' reversal on the normal diet (Table I). Microscopic evaluation of collagen content recorded as 1 to 4 plus closely paralleled the chemical findings.

*Effect of Ligation of the Portal Vein on the Collagen Content of Normal Liver and Livers with Cirrhosis Due to Carbon Tetrachloride*

Ligations of the portal vein were performed on 12 normal control rats. The animals were autopsied 34 to 42 days after the second stage of the operation. All showed adhesions between the liver and adjacent struc-

TABLE II  
*Composition of Diets Employed in Reversal of Cirrhosis  
Due to Carbon Tetrachloride*

Ingredient	Diet A	Diet B*	Diet C	Diet D	Diet E
	Low protein	Low protein	High protein	Low protein, high fat	High carbohydrate, low fat
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Casein	4	4	56	4	18
Cornstarch	30	30	4	22	33
Dextrose	30	30	4	22	33
Crisco	22	22	22	38	2
Cod-liver oil	2	2	2	2	2
Salt mixture	4	4	4	4	4
Yeast powder no. 300†	8	8	8	8	8

\* Supplemented with methionine, 500 mg.; choline, 125 mg.; and l-cystine, 312.5 mg. per 100 gm. of diet.

† Anheuser-Busch.

tures, including the intestine, omentum, diaphragm or anterior abdominal wall. The adhesions were marked in some, and minimal or slight in others. The average collagen content for this group was 0.30 per cent wet weight, as compared to the normal of 0.16 per cent. The average for hepatic collagen was 25 mg. per liver as compared to 18.6 mg. in normal livers. Microscopically, increased amounts of reticulum were visible (Fig. 7). Some of this increase was due to condensation of the normal stroma, since the livers in this group showed atrophy, their weights being less than those of normal controls and lower than the normal weight of the rat's liver according to Webster, Liljegren, and Zimmer.<sup>4</sup> The increase in the average total hepatic collagen following ligation of the portal vein, in spite of atrophy of the liver, indicates an absolute as well as a relative increase in collagen.

The portal vein was ligated in a group of 12 rats with hepatic cirrhosis, obtained from the same original large group of rats with cirrhosis due to carbon tetrachloride. The first stage of the operation was performed on the day after the carbon tetrachloride was discontinued, and the

second stage was done 3 days later. The rats were then autopsied in groups of 4 on the 16th, 23rd, and 36th day after the second operation. The average content of hepatic collagen in these groups was 0.35, 0.35, and 0.33 per cent, respectively. The only animal which showed no adhesions to the liver (Fig. 6) had the highest percentage of collagen of the entire group, 0.52 per cent. No correlation was apparent between the number or size of the adhesions and the amount of cirrhosis which persisted.

### DISCUSSION

Involutionary changes became apparent in the fibroblasts as reversal of the hepatic cirrhosis progressed (Fig. 2). Most of the fibroblasts were located in the fibrous trabeculae, and, as the latter became narrowed (Figs. 3 and 4), the cellular as well as the fibrillar elements became scarcer. Save for a gradual disappearance of reticular and collagenous fibers, no microscopic morphologic or staining peculiarities were seen in the connective tissue which was undergoing resorption. Occasional macrophages containing nuclear debris were identified in livers during the reversal phase, but no phagocytosis of fibrils or evidence of localized erosive action by phagocytes was observed.

The beneficial effect of supplemental methionine, choline, and cystine upon the reversal of cirrhosis on a low protein diet is most probably a result of the favorable influence which these substances exert upon the liver cells themselves, the cystine being beneficial only in small amounts to supplement a low protein intake.<sup>5,6</sup> An opposite and deleterious effect was seen when hepatic cells were damaged with a high fat, low protein diet, or when the portal vein was ligated. Although high fat, low protein diets produce hepatic cirrhosis, new deposition of fibrous tissue due to this diet does not occur during the short interval for which it was employed in the present study.<sup>7</sup> The persistence of cirrhosis following successful ligation of the portal vein is in accord with Mann's<sup>8</sup> demonstration that regeneration of the liver following partial hepatectomy is considerably impaired when no portal venous flow is present. It appears likely, then, that the disappearance of collagen from cirrhotic livers is closely related to, and is dependent upon, the functional state or regenerative activity of the liver cells themselves.

As previously noted,<sup>1</sup> the chemical values obtained by Lowry's method correlate best with microscopic evaluations which are based upon reticulum staining, rather than upon the trichrome method. This finding was confirmed in the present study, especially with regard to the increased reticulum which was observed following ligation of the portal vein. In these livers, the trichrome stain showed no appreciable deposit

of collagen, but parallel increases were seen in both the chemical values and in the amount of reticulum which was visible microscopically.

### SUMMARY AND CONCLUSIONS

Hepatic cirrhosis was produced in a group of 234 rats by exposure to carbon tetrachloride vapors every other day for 35 days.

The increase in collagen, as determined chemically, followed a curve of exponential type. Decrease in the collagen content occurred after stopping the carbon tetrachloride.

Reversal of cirrhosis was very nearly complete, and equally so with a normal diet, or with a low protein diet supplemented with methionine, choline, and cystine. Recovery from cirrhosis was impaired by a high fat, low protein diet.

Ligation of the portal vein also retarded recovery from cirrhosis, unless hepatic adhesions were present. The latter favored reversal of the cirrhosis.

Resorption of collagen from livers with cirrhosis depends upon the functional state or regenerative activity of the hepatic cells themselves.

Comparison of microscopic and chemical observations indicates that the method of Lowry determines reticulum as well as collagen.

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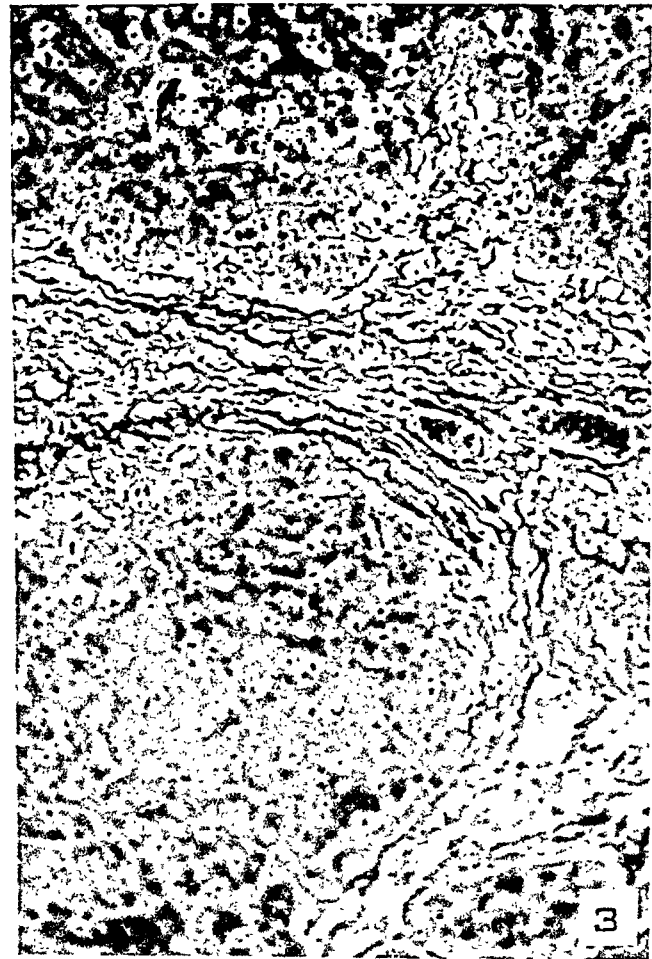
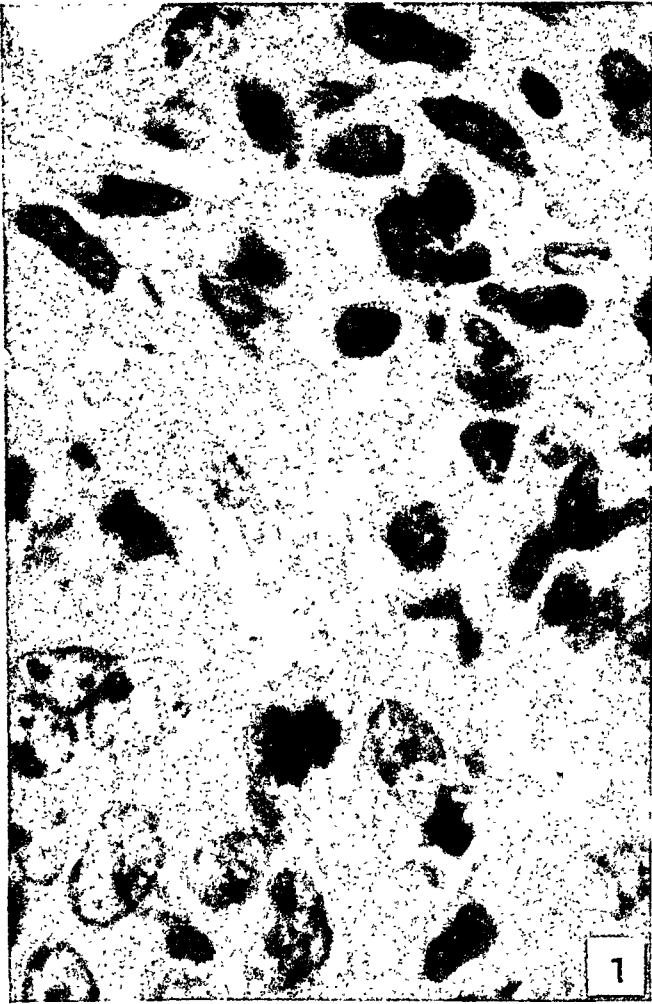
[ *Illustrations follow* ]

## DESCRIPTION OF PLATES

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### PLATE 36

- FIG. 1. Active proliferation of fibroblasts and bile duct epithelium in hepatic cirrhosis due to exposure to carbon tetrachloride for 20 days. The larger cells of a bile duct at the lower left corner are distinguishable from the plump, irregular, ovoid, fibroblastic nuclei elsewhere. Hematoxylin and eosin stain.  $\times 1000$ .
- FIG. 2. Cirrhosis due to carbon tetrachloride 14 days after stopping the drug. The fibroblastic nuclei, in comparison to those seen in Figure 1, are shrunken and pyknotic. Hematoxylin and eosin stain.  $\times 1000$ .
- FIG. 3. Cirrhosis due to exposure to carbon tetrachloride for 30 days. Fibrillar collagen and reticulum form broad septa. Laidlaw's reticulum and van Gieson's stains.  $\times 200$ .
- FIG. 4. Reversal of cirrhosis 30 days after discontinuing carbon tetrachloride. There is considerable disappearance of collagen and reticulum, with marked narrowing of the connective tissue septa as compared to Figure 3. Laidlaw's reticulum and van Gieson's stains.  $\times 200$ .



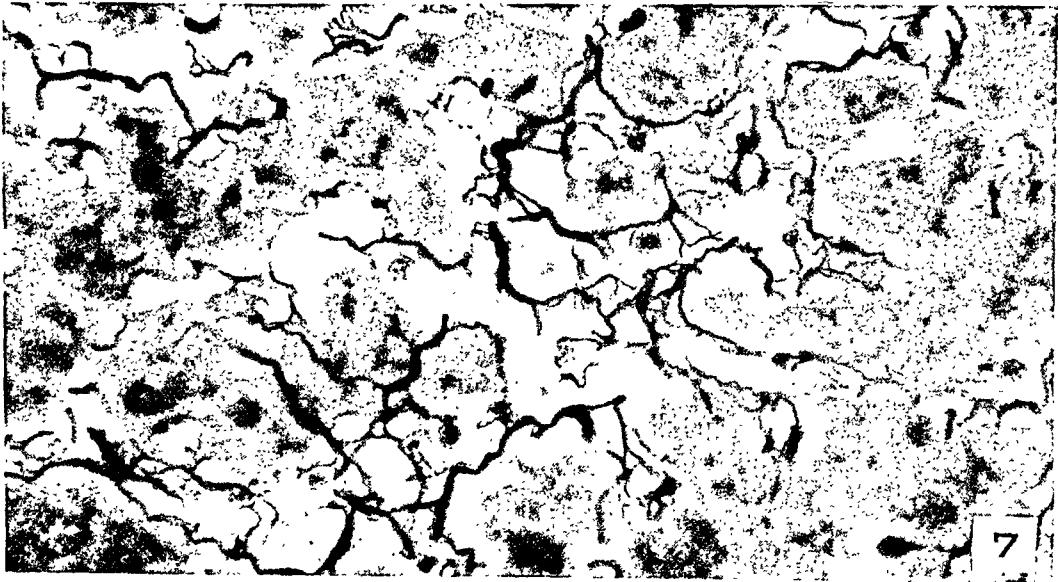
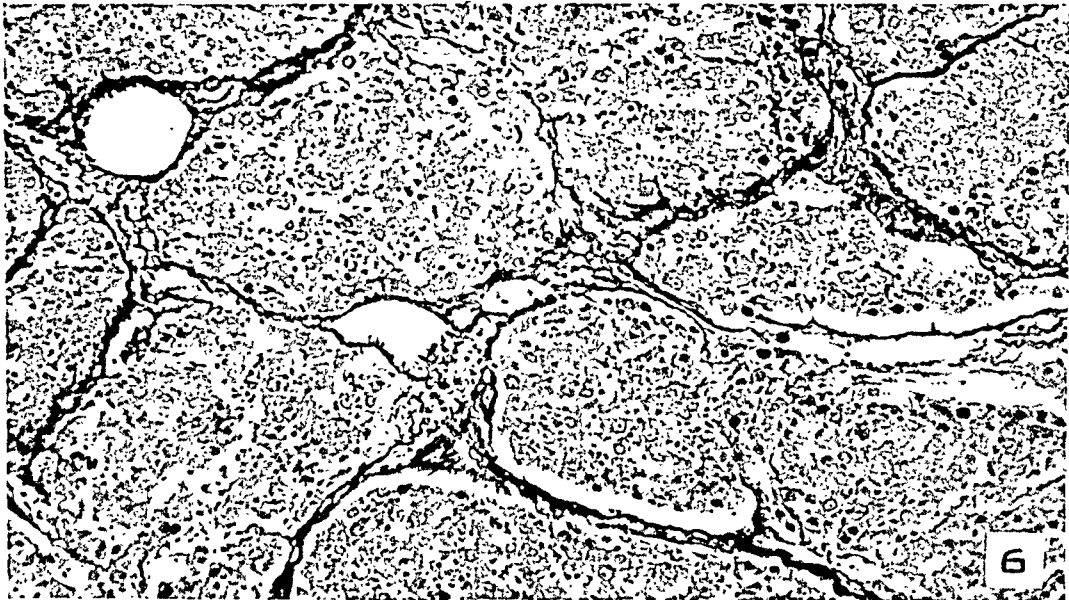
Merriene

Cellagen Content in Experimental Cirrhosis



PLATE 37

- FIG. 5. Persisting cirrhosis 38 days after stopping the administration of carbon tetrachloride. Animals were fed a low protein, high fat diet during the period of reversal. Collagen content was 0.68 per cent. Laidlaw's reticulum and van Gieson's stains.  $\times 175$ .
- FIG. 6. Failure of reversal of carbon tetrachloride cirrhosis in a rat with ligation of the portal vein. Collagen content was 0.52 per cent 23 days after stopping the drug. The portal vein was ligated after the cirrhosis had been produced. No hepatic lesions were present. Laidlaw's reticulum and van Gieson's stains.  $\times 175$ .
- FIG. 7. Increased reticulum 42 days following ligation of the portal vein in a normal rat. Collagen content was 0.35 per cent. Laidlaw's reticulum and van Gieson's stains.  $\times 500$ .





## CUTANEOUS LEIOMYOMA OF GOLDFISH

### I. MORPHOLOGY AND GROWTH IN TISSUE CULTURE \*

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Approximately 3 to 4 per cent of the goldfish in a 3-acre urban pond have been found to bear single or multiple cutaneous tumors. The fish averaged 25 cm. in length; a study of their scales showed that the majority were about 5 years old. They appeared healthy, although an occasional specimen either with or without a tumor was infested with copepods of the species *Lernaea carassi* (Tidd). The pond was created 20 years ago by damming a small stream; at present most of the water is supplied by a spring. The fishes, including goldfish, sunfish, and minnows, are descendants of those originally used to stock the pond. Only the goldfish bore cutaneous neoplasms.†

#### *Gross Examination*

The descriptions of the gross and microscopic appearance of these tumors are based upon the study of material obtained from 14 goldfish (*Carassius auratus*) that were kept under observation in aquaria for periods ranging from 45 to 203 days. When small, the tumors were moderately firm and orange-yellow. They arose at the base of a scale, growing over and burying it as they increased in size; the free surfaces were covered by epidermis. As the neoplasms became larger they remained broadly sessile, were softer, and sometimes displayed several areas of cystic degeneration. The surface was smooth, but in the large growths it was sometimes faintly lobulated or even ulcerated. On section there was profuse bleeding; the cut surface was homogeneous and gray-pink.

The tumors occurred in widely scattered regions of the trunk as well as upon the head and all the fins. Although no area of predilection has been observed, three of the largest and most rapidly growing neoplasms arose on the trunk immediately behind the operculum (Fig. 1). Such large tumors usually were single, although some were accompanied by 2 or 3 small nodules on the trunk or fins. Multiple growths (5 to 10) usually were small, seldom over 15 mm. in greatest diameter (Fig. 2).

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† Permission to remove these fish from the pool on the grounds of The Cleveland Museum of Art was given by the Director, Mr. W. M. Milliken.

These multiple lesions appeared to be multicentric rather than metastatic in origin. In none of these fish examined has there been any evidence of visceral metastasis.

### *Microscopic Examination*

Histologically, the tumors were composed of long spindle cells arranged in broad interlacing bundles (Fig. 3). The surface epithelium rested directly upon the neoplastic cells and might send long pegs into the tumor, particularly when the growth was a small one (Fig. 4). Areas of myxomatous degeneration were found in which the cells appeared bipolar. In a large, slowly growing tumor on the caudal fin, one portion of the lesion showed palisading of the nuclei (Fig. 5), but the Bodian stain failed to reveal any trace of nerve fibrils. Van Gieson's stain and Milligan's trichrome stain<sup>13</sup> gave the reaction characteristic of smooth muscle rather than of connective tissue in all of the tumors. Myofibrils could be demonstrated in at least some of the cells in every neoplasm.

In the large, rapidly growing tumors many of the cells had lost their elongate character and were broad polygonal elements with abundant polychromatic cytoplasm and large oval nuclei that contained 2 to 4 prominent nucleoli (Fig. 6). These cells at times reverted to the characteristic spindle shape when the growth rate was reduced. This was demonstrated by the response of one of the tumors to transplantation. When a tumor fragment was placed in the anterior chamber of the right eye as an autotransplant, it grew slowly for a period of 3 months. At the end of that time the animal died; sections of the eye showed that the transplant had assumed the pattern of less rapidly growing tumors.

Occasionally, the large polygonal cells contained acidophilic intracytoplasmic inclusions (Fig. 7). Whether these merely represented foci of degeneration, or were indicative of the presence of a virus has not been determined. Multinucleated tumor giant cells were seen infrequently, but in some sections of the rapidly growing neoplasms they were a prominent element in a few areas (Fig. 8).

### GROWTH IN TISSUE CULTURE

Attempts at *in vitro* culture of the tumor cells were successful with each of three large, rapidly growing tumors. A fourth tumor of this kind showed consistent contamination with a chromogenic bacterium, probably *Pseudomonas fluorescens*. Another large but stationary neoplasm, described above as showing palisading of nuclei in histologic sections, failed to grow in tissue culture. An effort was made also to cultivate small, slowly growing tumors *in vitro*, but without success.

### Methods

The tissues were cultured at room temperature (19° to 23°C.) using the hanging-drop method and the roller tube technic of Gey.<sup>14</sup> The medium was the same as that most often employed for mammalian tissues: chicken plasma and beef or chick embryo extract for clot, with blood serum from human umbilical cord and Tyrode's saline solution as the liquid phase. The fluid in the roller tubes was changed every second day. Serial transplantation was not effected, but single cultures were kept under observation for as long as 8 weeks. To guard against infection, the surface of the tumor was first cut away and the tissue for explantation then removed and washed in several changes of Tyrode's saline solution. Like several other investigators, I employed penicillin to the amount of 1500 units per cc. in the Tyrode's solution used in the liquid medium. After several days, during which growth had begun, penicillin often was omitted from some of the cultures, but no effect on the pattern or rate of growth was observed.

Grand, Gordon, and Cameron<sup>1</sup> found that the melanoblastoma of a hybrid tropical aquarium fish grew better *in vitro* when fish serum was used in place of human cord serum. A similar effect was not apparent with the goldfish tumors when the blood of carp, a species very closely related to the goldfish, was used instead of human serum. Evidence of the remarkable indifference of goldfish tissue to the source of the culture media is shown by the behavior of adult heart muscle in media derived from human and bovine sources. Not only did the explants show active outgrowth of fibroblasts, but 18 days after transplantation the muscle began to beat rhythmically at the rate of once every 15 seconds. This ceased 6 days later, but the growth of fibroblasts continued.

### Observations

On the second day after transplantation of the neoplastic tissue to a hanging-drop or roller tube, macrophages had migrated some distance from the explant. These wandering cells were identified by their almost circular outline, and by the presence of many highly refractile granules in the cytoplasm. After this emigration of the macrophages, large flat cells were seen growing out from the periphery of the explant. These had very finely granular or clear cytoplasm and large oval nuclei which after fixation were pale staining, with 2 to 4 nucleoli (Fig. 9). The cells might fuse to form multinucleated giant cells which resembled those seen in sections of the tumor (Fig. 10). Mitotic figures occasionally were observed; rarely, they were tripolar.

The general pattern of growth was that of a sheet of cells spreading

out from the explant, and was best seen in roller tube cultures (Fig. 11). The large cells were the predominant element in all cultures, whether growing in a hanging-drop or roller tube, and undoubtedly represented the neoplastic component. They bore a striking resemblance to the large cells seen in histologic sections of the rapidly growing primary tumors (Fig. 6).

Although the cytoplasm in the living cells usually was quite structureless, myofibrils sometimes were seen in the fixed and stained preparations. Tension striae (Fig. 12) were prominent in the large flat cells of some hanging-drop cultures. In the latter, occasional cells contained acidophilic cytoplasmic inclusions (Fig. 13). The significance of these structures has not been evaluated and the possibility that they represent merely phagocytized protein cannot be overlooked. The resemblance which they bear to similar inclusions found in the tissue sections is striking (Fig. 7).

In several of the hanging-drop preparations there were many cells with hyperchromatic nuclei and condensed cytoplasm forming a fibrillated strand which lent the cell a very long spindle shape or even a bipolar character (Fig. 14). A cytoplasmic vacuole which deformed the nucleus was of frequent occurrence. When examined stereoscopically, the relationship between these cells and the large flat ones became clear. Long spindle cells were the only type found in the depth of the culture, where they were surrounded by coagulum. When one of these cells came in contact with the overlying coverglass, the cytoplasm spread out in a broad thin film; the nucleus, too, became flattened and the chromatin appeared less dense. The cells had then become indistinguishable from the large polygonal cells described above.

As early as 90 hours after explantation, sprouts of endothelium appeared in the proliferating zone of cells (Fig. 10). Behind the growing tip, a lumen separated the adjacent cells, producing a rather well formed capillary. These could always be traced back to the explant and were outgrowths from capillaries present in the tumor at the time of transfer. After several weeks these channels might form an extensive branching network. The caliber of the lumen was fairly uniform and usually smaller than, although occasionally equal to, that observed in the cutaneous capillaries of the living fish.

As previously noted, the cultures were kept at room temperature which varied between 19° and 23° C. Twelve actively growing roller tube cultures were placed in an incubator at 37° C. Growth continued for 3 days but thereafter ceased and regressive changes set in. During the next 17 days a control group kept at room temperature continued

to grow, while those in the incubator reached advanced stages of degeneration. When returned to room temperature these cultures failed to initiate new growth.

### DISCUSSION

Tumors in fishes are not very uncommon, but in view of the widespread use of goldfish as pets and for ornamental purposes, it is rather surprising that only 13 reports of neoplasms in these fish are found in the literature.<sup>2</sup> In 10 the lesions were identified as fibromas or fibrosarcomas of the corium or subcutaneous connective tissue. Although there is no record of leiomyomas in goldfish, it is possible that some of the tumors identified as of connective tissue origin were in fact derivatives of smooth muscle.

The question of the benign or malignant nature of the leiomyomas is not easily answered. Certainly the small, slowly growing, usually multiple neoplasms must be classified as benign. The large, rapidly growing tumors display many characteristics of malignant lesions; *e.g.*, many mitotic figures, large nuclei with prominent nucleoli, and invasion of surrounding tissues. Metastases were not observed, but these are uncommon in fishes; the entire literature contains only 14 instances in which a primary tumor had metastasized,<sup>2</sup> none being reported in goldfish. The reason for this absence of metastasis is obscure and the explanation commonly given, *viz.*, that the tumor-bearing and therefore handicapped fish are destroyed before the lesion has had time to metastasize, is scarcely pertinent in this instance, since the animals were carefully tended in aquaria while the tumors increased rapidly in size.

Although the neoplastic smooth muscle cells were grown readily *in vitro*, at no time did they show evidence of contraction. That the media used permitted development of normal structure and function is indicated by the resumption of rhythmic contraction by a bit of adult goldfish heart 18 days after explantation. The absence of contraction may be attributed in part to the fact that most of the smooth muscle cells were spread out flat against the coverglass. In her study of smooth muscle cells grown from chick amnion, M. Lewis<sup>3</sup> observed that the flattened cells usually failed to contract; whereas, she saw occasional rhythmic contractions in the elongated and band-like cells near the explant.

Myofibrils were not observed in the living tumor cells and could be identified only with difficulty in the fixed and stained preparations. M. Lewis<sup>3</sup> also was unable to recognize myofibrils in living smooth muscle cells cultured from chick amnion, although they were readily visible after fixation. Similar findings are recorded by W. Lewis<sup>4</sup> in



his study of embryonic chick heart explants, which frequently beat rhythmically in the absence of both cross and longitudinal striations. Champy,<sup>6</sup> employing urinary bladder and arterioles of the adult rabbit, found that the smooth muscle cells present in his cultures did not contract and were without fibrillae even after fixation; therefore they were indistinguishable from fibroblasts. Bloom<sup>6</sup> has suggested that the use of adult tissues by Champy and of embryonic tissues by the Lewises accounts for the discrepancies in their results. The neoplastic smooth muscle cells of fish bear a striking resemblance to those of the amnion of the chick in size and shape, and in the presence of occasional fibrillae after fixation. Their lack of functional activity more closely resembles that of cells of the adult rabbit which likewise failed to contract.

The tension striae seen in some of the cells (Fig. 12) are similar to those described by the Lewises,<sup>7</sup> not only in heart and smooth muscle cells, but also in endothelium and mesothelium. The striae appear to be folds in the surface of the cell produced by tension and reversible when this is relaxed. The effect upon the cell of surface forces acting at the interface of glass and cytoplasm is indicated not only by the presence of tension striae but by the large flat shape which the cell assumes. When growing in fibrin clot these same cells appear as elongated spindles (Fig. 14).

The source of the neoplastic smooth muscle cells has not been identified with certainty, but is probably the walls of blood vessels. This site has been accepted by Stout<sup>8</sup> for the majority of solitary cutaneous leiomyomas in man. In the multiple cutaneous leiomyomas reviewed by Ormsby,<sup>9</sup> the tumors arose chiefly in the arrectores pilorum. Stout pointed out that the human cutaneous leiomyomas may develop in the walls of larger veins but not of arteries. He believed that the tumor vessels are neither normal veins nor normal arteries, and that muscle bundles of the vessels merge with the neoplastic muscle so frequently that the origin of the latter from the former seems evident.

The corium of the goldfish is very vascular, with surprisingly large arterioles present just below the epidermis. The muscle in the walls of these vessels closely resembles that seen in the tumors. Search for arteriovenous shunts in sections of the corium as well as in the caudal fin of living fish has been fruitless. In 1888 Mayer<sup>10</sup> gave a detailed account of peculiar doughnut-shaped, isolated masses of smooth muscle that surround arterioles in the subcutaneous tissue and viscera of certain selachians, especially the skate *Raja clavata*. No similar structures were seen in the corium of goldfish, nor could a report of their occurrence in teleost fishes be found in the literature.

All of the tumor-bearing goldfish described in this report have been obtained from the same pond. Many more fish were observed with tumors than were collected; an effort is being made to preserve this stock for further observations on its life history and ecology. Despite careful inquiries among local aquarists and the Ohio State Department of Conservation, no other pools with tumor-bearing goldfish could be located in this vicinity. The rather high incidence of this unusual tumor in one species of fish in a single pond points to an extrinsic agent acting perhaps on a highly susceptible inbred strain. The occasional cytoplasmic inclusions observed in the tumor cells lend support to this possibility.

An infectious etiology for certain tumors of goldfish has been suggested by two groups of investigators. In a tank containing 20 goldfish, Roffo<sup>11</sup> found 7 with neoplasms that he identified as fibrosarcomas. He found proof of the infectious character of the tumor in the fact that neoplasms did not develop among goldfish placed in the tank after it had been sterilized and the diseased animals removed. Montpellier and Dieuzeide,<sup>12</sup> in their report of fibrosarcomas in each of 5 goldfish from the same pool, also raised the question of an infectious agent.

#### SUMMARY

Approximately 3 to 4 per cent of the goldfish in a large pond bore single or multiple leiomyomas.

The tumors probably arose in the walls of blood vessels and some reached a diameter of 5 cm. Such large, rapidly growing neoplasms were locally invasive and histologically malignant. No metastases have been found.

The general pattern of growth in tissue culture was that of a sheet of cells spreading from the explant. Large flat cells fused to form giant cells similar to those seen in sections.

Acidophilic inclusions were present in the cytoplasm of occasional tumor cells; these were seen in histologic sections of the neoplasms as well as in cells growing *in vitro*.

It is suggested that these tumors may be due to an infectious agent acting on a highly susceptible inbred strain of fish.

Technical assistance in the preparation of the tissue cultures and photographs by Miss Roberta Walker and Mrs. Ralph Lewis is gratefully acknowledged.

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## DESCRIPTION OF PLATES

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### PLATE 38

- FIG. 1. A rapidly growing tumor on the trunk, directly behind the operculum. The fish had been under observation for 57 days, during which time the tumor doubled in size.
- FIG. 2. Multiple leiomyomas are present on the trunk, caudal and dorsal fins, and operculum.
- FIG. 3. Section illustrating the characteristic pattern of interlacing bundles assumed by the spindle-shaped tumor cells. Milligan's trichrome stain.  $\times 275$ .

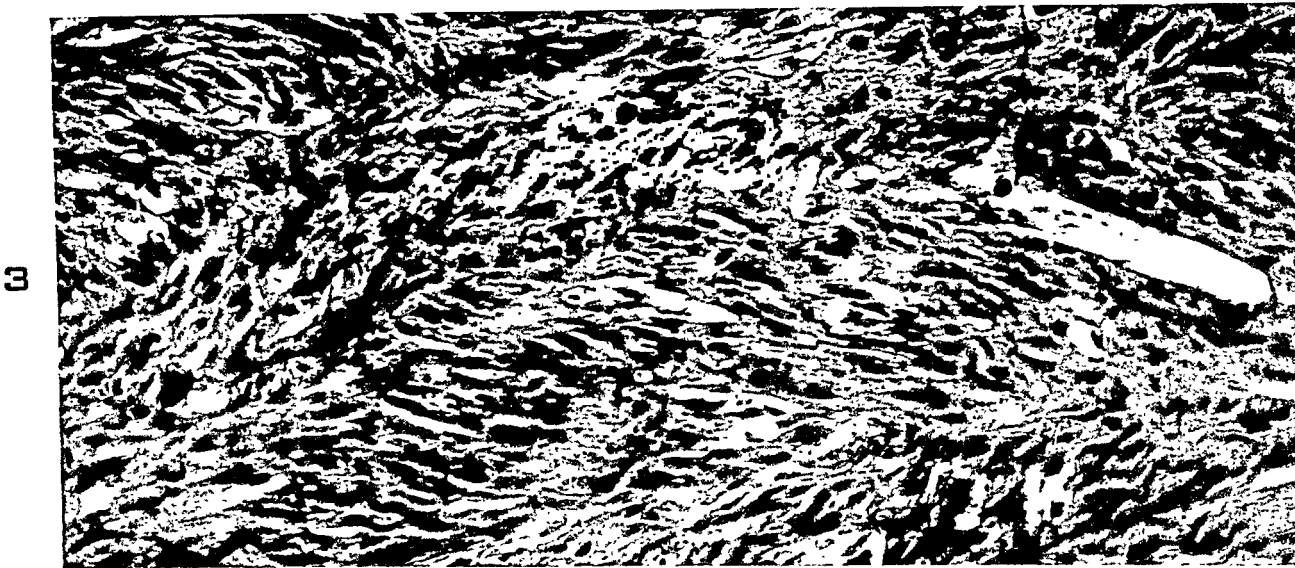
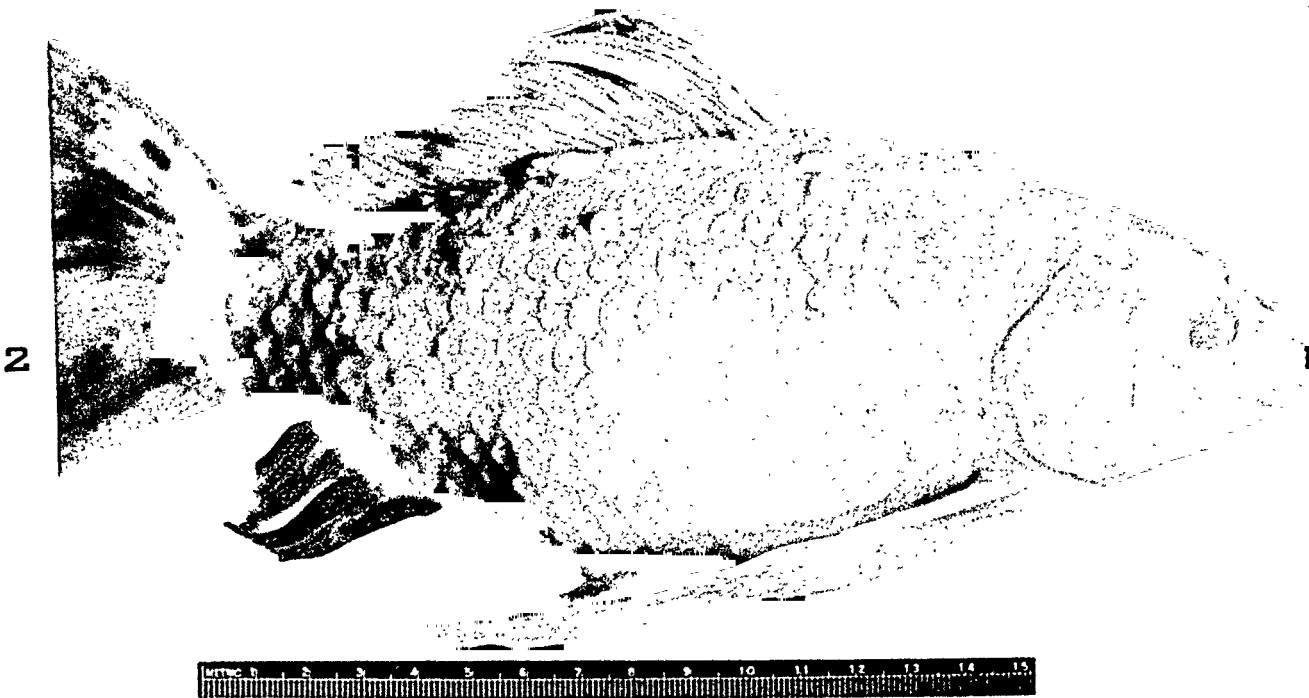
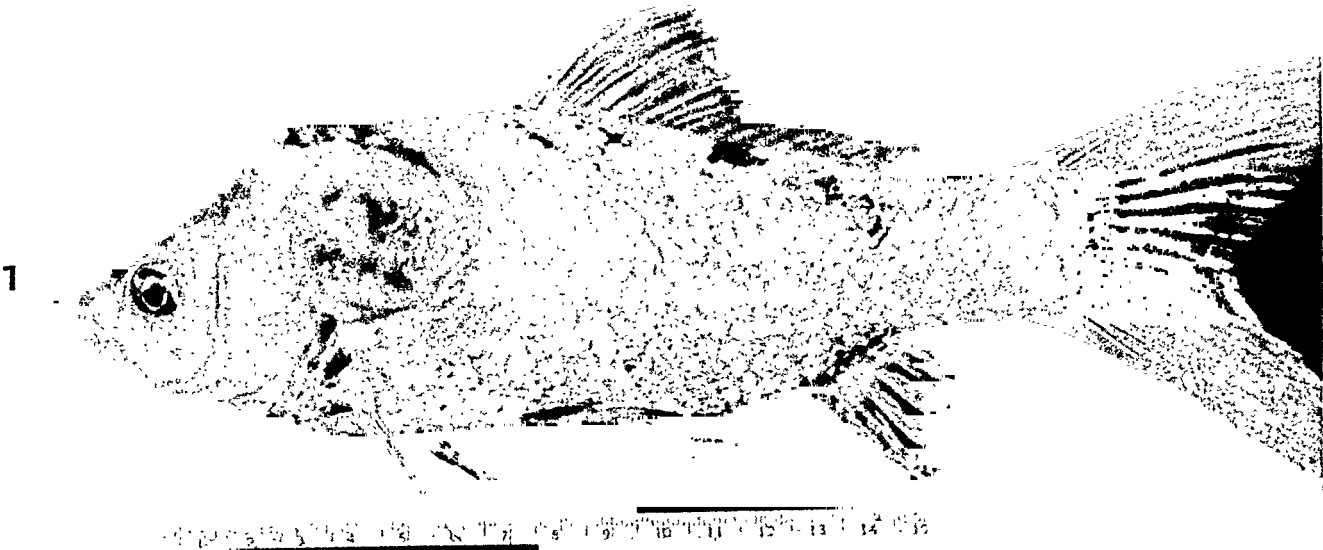


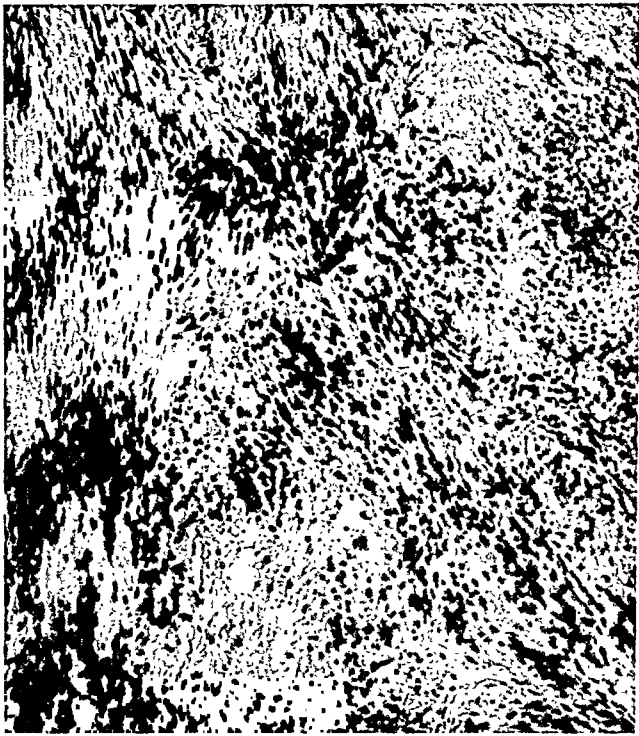
PLATE 39

- FIG. 4. Section through a small cutaneous nodule showing two epithelial pegs extending deeply into the tumor. The neoplastic smooth muscle cells are in intimate contact with the epidermis. Myxomatous degeneration, lending many of the cells a bipolar character, is prominent. Hematoxylin and eosin stain.  $\times 100$ .
- FIG. 5. Palisading of the nuclei is present in one portion of a slowly growing tumor, 2.5 by 2 by 2 cm., on the caudal fin. The Bodian stain failed to reveal any trace of nerve fibrils. Hematoxylin and eosin stain.  $\times 150$ .
- FIG. 6. Section of a rapidly growing tumor, 3.5 by 2.5 by 2 cm., on the dorsum of the head. The cells possess an abundant polychromatic cytoplasm, are in direct contact with the epidermis, and contain large nuclei with finely dispersed chromatin. Hematoxylin and eosin stain.  $\times 275$ .
- FIG. 7. Acidophilic cytoplasmic inclusion body in a cell from the tumor shown in Figure 6. Seller's basic fuchsin stain.<sup>15</sup>  $\times 950$ .
- FIG. 8. Multinucleated tumor giant cells. Hematoxylin and eosin stain.  $\times 700$ .

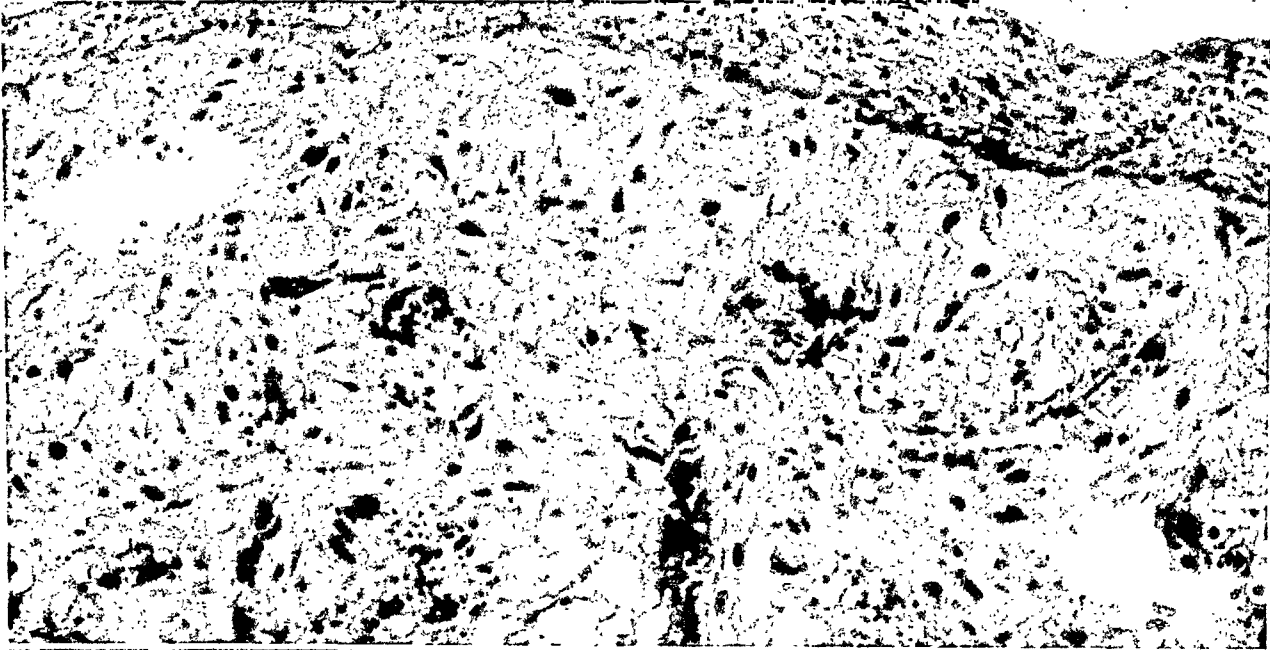
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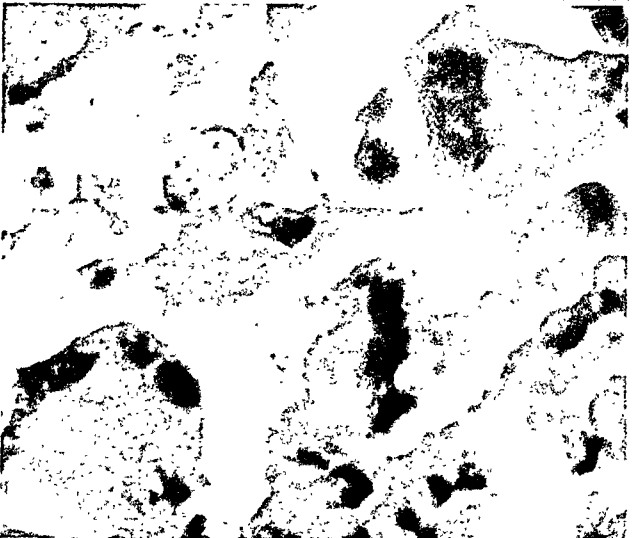
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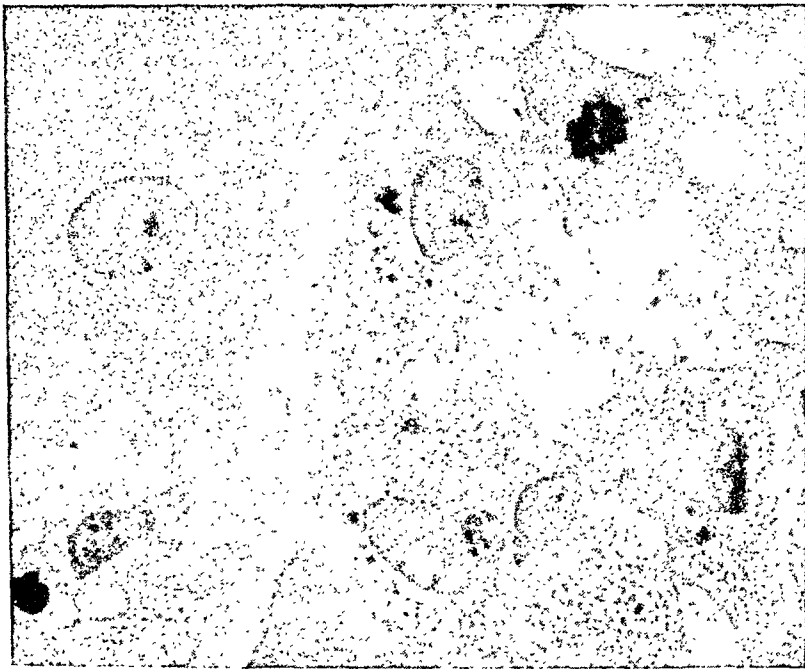
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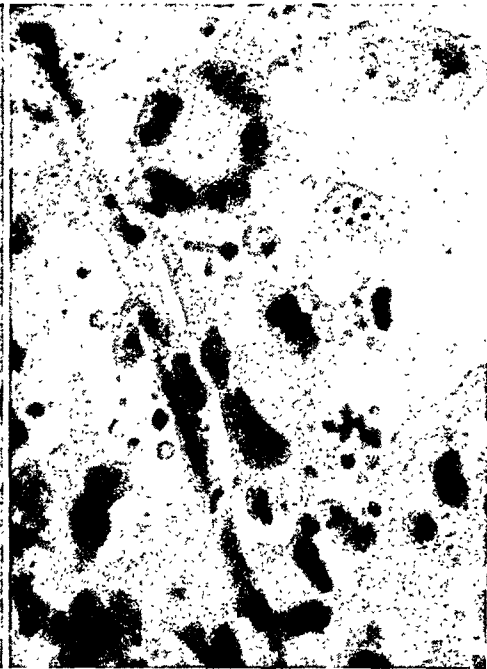
## PLATE 40

- FIG. 9. Characteristic appearance of tumor cells when in contact with the coverglass. There is an abundant granular cytoplasm; in the living cell such coarse granularity is not present. The chromatin is finely dispersed within the nuclei. In the upper right corner of the photomicrograph a cell is undergoing mitosis. Hanging-drop culture, 162 hours after transplantation. Hematoxylin and eosin stain.  $\times 700$ .
- FIG. 10. A strand of endothelial cells passes diagonally across the field. Touching it at one point is a circlet of nuclei which may represent a tumor giant cell. Multiple, linearly arranged nucleoli are clearly visible in the nuclei. Hanging-drop culture, 90 hours after transplantation. Hematoxylin and eosin stain.  $\times 500$ .
- FIG. 11. Sheet-like outgrowth of tumor cells in a roller tube culture, 8 days after transplantation. Hematoxylin and eosin stain.  $\times 80$ .
- FIG. 12. Tension striae in the cytoplasm of neoplastic smooth muscle cells, 90 hours after transplantation. Hanging-drop culture. Hematoxylin and eosin stain.  $\times 500$ .
- FIG. 13. Acidophilic cytoplasmic inclusions in tumor cells grown for 90 hours in a hanging-drop culture. Hematoxylin and eosin stain.  $\times 700$ .
- FIG. 14. The cells that are no longer in contact with the coverglass have acquired a very long spindle shape somewhat suggestive of bipolar nerve cells. However, transition stages between these and the large flat polygonal cells are numerous. Hanging-drop culture, 162 hours after transplantation. Hematoxylin and eosin stain.  $\times 350$ .

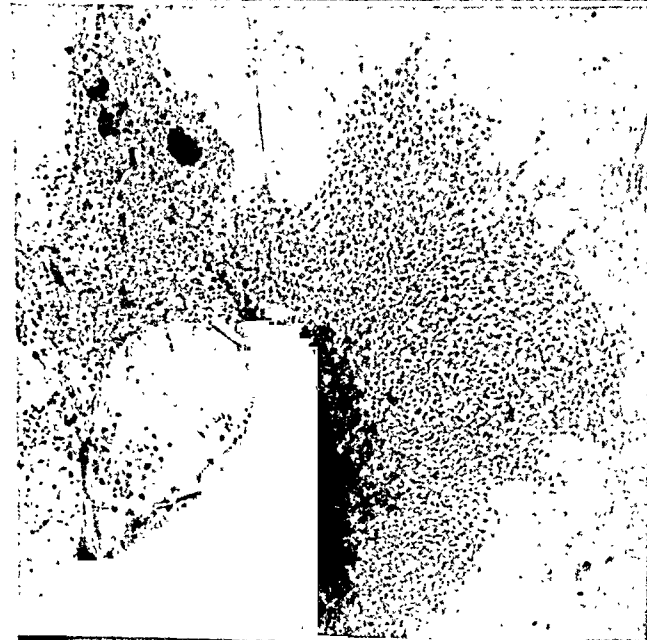
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# THE EFFECTS OF DEPRIVATION OF WATER ON THE ADRENAL GLANDS OF RATS \*

JOHN NICHOLS

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In recent years many papers have appeared dealing with the reaction of the adrenal gland to various forms of physiologic stress. So far as I can ascertain, there have been no published reports on the effects of acute dehydration. It was with this in mind that the following study was undertaken.

## MATERIALS AND METHODS

Sixteen female albino rats of the Wistar strain were used. These rats weighed about 300 gm. each, with none varying more than 10 gm. Five were killed with chloroform and designated as normal. The remainder were kept in screen-bottomed cages and allowed access to purina laboratory chow at all times. No water was allowed. On the 8th day 4 animals were killed with chloroform. One rat died on the 9th day, 4 on the 10th day, and one each on the 11th and 12th days.

When each animal died or was killed the adrenal glands were removed immediately and immersed in 10 per cent formalin. After fixation for 24 hours they were rinsed in tap water, and the surrounding fat was carefully dissected away. One gland from each animal was used for histologic study and the other for chemical determinations. The gland which was chosen for histologic study was embedded, cut by the freezing method, and mounted by the method of Zwemer.<sup>1</sup> For demonstration of total fats, the sections were stained with sudan III by the method of Romeis.<sup>2</sup> For demonstration of cholesterol content the Schultz reaction was used.<sup>3</sup> The method used with the other gland for the quantitative chemical determination of total fats and cholesterol has been described previously.<sup>4</sup>

## RESULTS

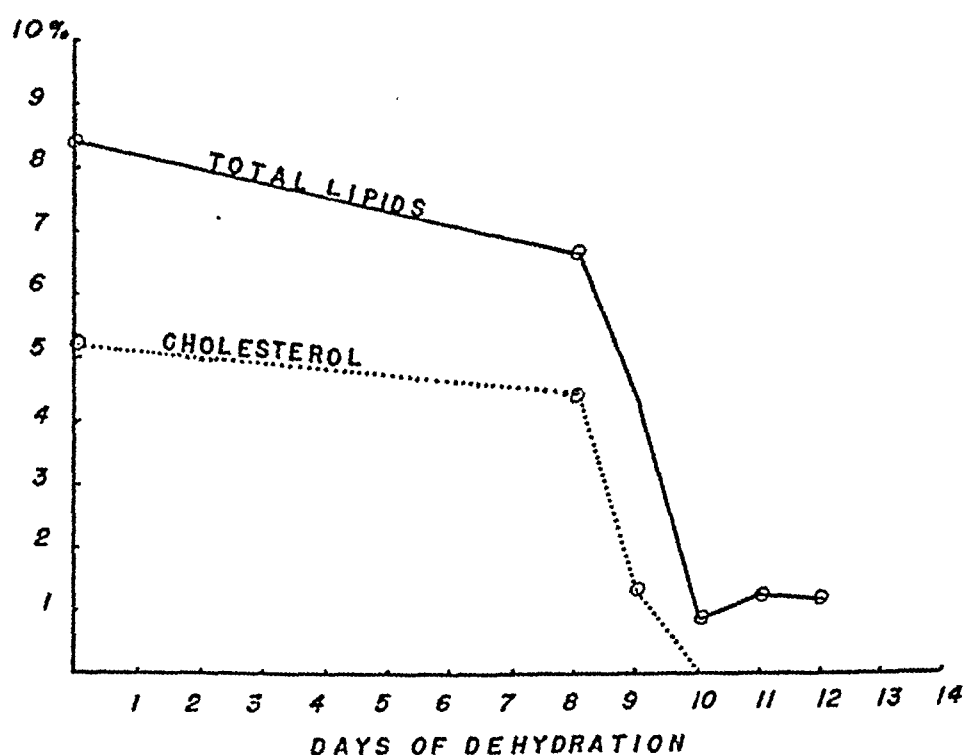
The results obtained from the quantitative analysis are tabulated and illustrated graphically (Text-Fig. 1). There was a marked decrease in total fats and cholesterol as dehydration proceeded. However, the weights of the glands and the water content were remarkably constant (Table I).

The histologic picture as revealed by the sudan III and the Schultz methods closely followed the quantitative findings. The distribution of total lipids in the normal gland is shown in Figure 1. Here may be seen a well outlined zona glomerulosa staining heavily with sudan; just inside

\* Received for publication, April 6, 1948.

there is a narrow, lightly staining zone. The zona fasciculata is also heavily stained, but the stain decreases in intensity as the zona reticularis is approached. The zona reticularis stains somewhat lighter than the zona fasciculata and it grades off until it reaches the medulla, where it ends abruptly. The Schultz reaction for cholesterol closely paralleled that of the sudan stain for total fats.

In the rats which had been dehydrated for 8 days there was found essentially the same condition in the adrenals as in the normal animals, except that the intensity of the sudan and Schultz stains was slightly less throughout.



Text-Figure 1. Depletion of total lipids and of cholesterol in the adrenals of rats deprived of water.

The rat which died on the 9th day showed marked changes (Fig. 2). In a section of the adrenal from this animal, stained with sudan for total fats, the zona glomerulosa is seen to be intact and well outlined although staining somewhat less densely than in the normal animals. The total lipids of the zona fasciculata and outer reticularis have become markedly depleted, while the inner reticularis is somewhat less disturbed. The Schultz reaction for the cholesterol fraction showed that the cholesterol had become depleted in the same manner and distribution as the total lipids.

The adrenals of the rats which died on the 10th, 11th, and 12th days showed the greatest change. Lipids were almost entirely absent in all

TABLE I  
The Weight and Lipid Content of the Adrenal Glands of Dehydrated Rats

Treatment	Rat no.	Wet weight, adrenal mg.	Dry weight, adrenal mg.	Water per cent	Ether-extracted weight mg.	Fat per cent	Cholesterol weight mg.	Cholesterol per cent
Normal	1	18.1	6.9	61	5.4	8.5	0.780	4.3
Normal	2	16.0	6.3	61	4.8	9.3	0.885	5.5
Normal	3	12.1	4.8	60	3.7	9.0	0.700	5.8
Normal	4	15.1	6.0	60	4.6	9.2	0.855	5.6
Normal	5	14.7	5.2	64	4.3	6.1	0.735	5.0
Average						8.4		5.2
Dehydrated	6	11.8	5.0	57	4.2	6.8	0.650	5.5
8 days	7	14.3	5.8	59	4.7	7.6	0.625	4.3
8 days	8	14.0	6.1	56	4.8	8.4	0.625	4.4
8 days	9	14.9	5.8	61	6.4	4.0	0.600	4.0
Average						6.7		4.5
Died of dehydration	10	18.5	7.4	60	6.6	4.3	0.250	1.3
9th day	11	21.2	7.9	62	7.8	0.5	Too low to read	colorimeter
10th day	12	17.6	6.4	63	6.2	1.0	Too low to read	colorimeter
10th day	13	20.5	8.1	60	7.9	0.9	Too low to read	colorimeter
10th day	14	20.1	8.2	54	7.9	1.4	Too low to read	colorimeter
11th day	15	22.1	8.7	60	8.4	1.3	Too low to read	colorimeter
12th day	16	15.4	5.8	62	5.6	1.2	Too low to read	colorimeter
Average						1.5*		

\* 1.05 per cent for 6 animals surviving 10 days or more.

of the zones, only a few lipid droplets remaining in the zona glomerulosa. The Schultz reaction revealed that the cholesterol had entirely disappeared. These glands are not illustrated but the chemical findings are indicated in Table I.

### DISCUSSION

Most investigators who have subjected animals to stresses of the general type considered here have found marked depletion of lipids of the adrenals, especially in the inner zones. Dosne and Dalton,<sup>5</sup> studying the effects of cold, fasting, and the injection of formaldehyde, obtained depletion of lipids, as did Oleson and Bloor<sup>6</sup> who studied fasting. Andersen,<sup>7</sup> and Knouff, Brown, and Schneider<sup>8</sup> obtained lipid depletion following or during increased muscular activity. Levin,<sup>9</sup> Langley and Clarke,<sup>10</sup> Giragossintz and Sundstroem,<sup>11</sup> Darrow and Sarason,<sup>12</sup> Dalton, Mitchell, Jones, and Peters,<sup>13</sup> Tepperman, Tepperman, Patton, and Nims,<sup>14</sup> and Nichols<sup>4</sup> obtained depletion of lipids in the inner zones during exposure to anoxia. Ludewig and Chanutin<sup>15</sup> found that prolonged chloroform anesthesia causes depletion of lipids. Vogt<sup>16</sup> found the same after injection of insulin. These reactions are considered by Selye<sup>17</sup> to be part of the "alarm reaction."

I am unable to give a definite explanation for the changes observed. However, there are certain reported observations which may be related. Marriott<sup>18</sup> has demonstrated that in the early stages of anhydremia there is an increase of blood proteins and in the advanced stages a decrease in blood proteins together with a high nonprotein nitrogen. Dougherty and White,<sup>19</sup> in a series of publications, have shown that the 11-oxycorticosteroids secreted by the zona fasciculata and reticularis cause a dissolution of lymphatic tissue and an increase of blood protein, especially in the gamma globulin fraction. Since 100 gm. of protein can give rise to 45 gm. of endogenous water, the changes in the adrenal described in this paper may be part of an attempt by the organism to provide itself with more water without the use of exogenous food. The fascicular and reticular zones, first affected in the experiments reported here, are known to be under the control of the pituitary gland (the zona glomerulosa is not). This has been demonstrated by Smith,<sup>20</sup> Crooke and Gilmour,<sup>21</sup> Sarason,<sup>22</sup> and many others, who found atrophy of these two inner zones after hypophysectomy. This atrophy can be prevented by administration of adrenotrophic hormone (Meyer, Mellish, and Kupperman<sup>23</sup>).

### CONCLUSION

Marked depletion of total lipids and especially of cholesterol was found in the adrenal cortices of rats subjected to prolonged dehydration. This depletion was especially pronounced in the zona fasciculata and

zona reticularis. The zona glomerulosa was the last to become depleted of lipids. The histologic observations were found to be closely paralleled by quantitative chemical findings.

#### ADDENDUM

It has recently been pointed out by Schmidt-Nielsen, Schmidt-Nielsen, and Schneiderman<sup>24</sup> that certain desert mammals, especially the kangaroo rat (*Dipodomys merriami*), do not require water; in fact these animals can live indefinitely on desiccated grain. Furthermore, these animals can excrete urine with a 0.908 normal chloride concentration. This is one and one-half times the normal maximum for the laboratory rat and three times that of man. Experiments are being carried out on this animal in this laboratory.

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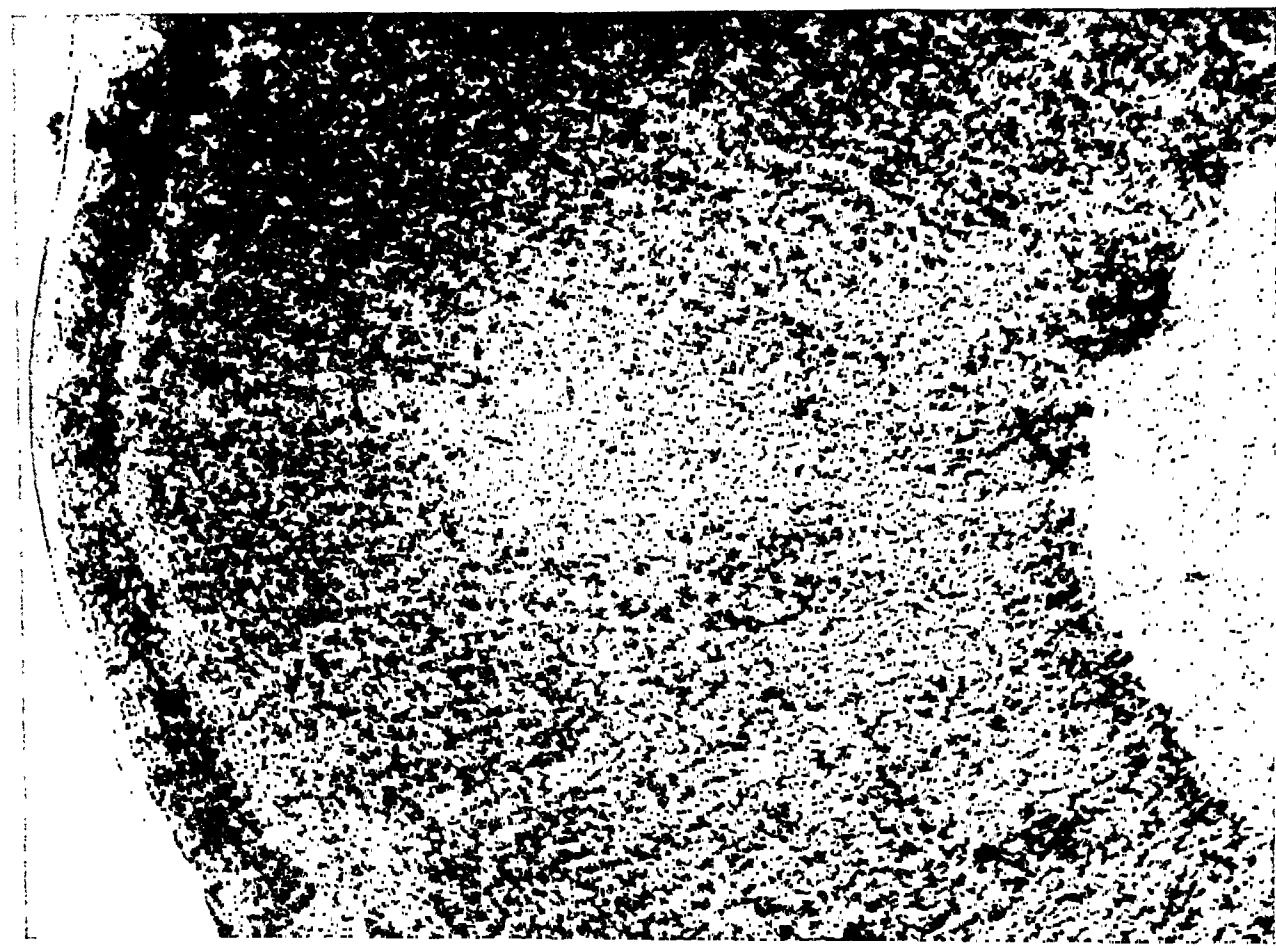
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### DESCRIPTION OF PLATE

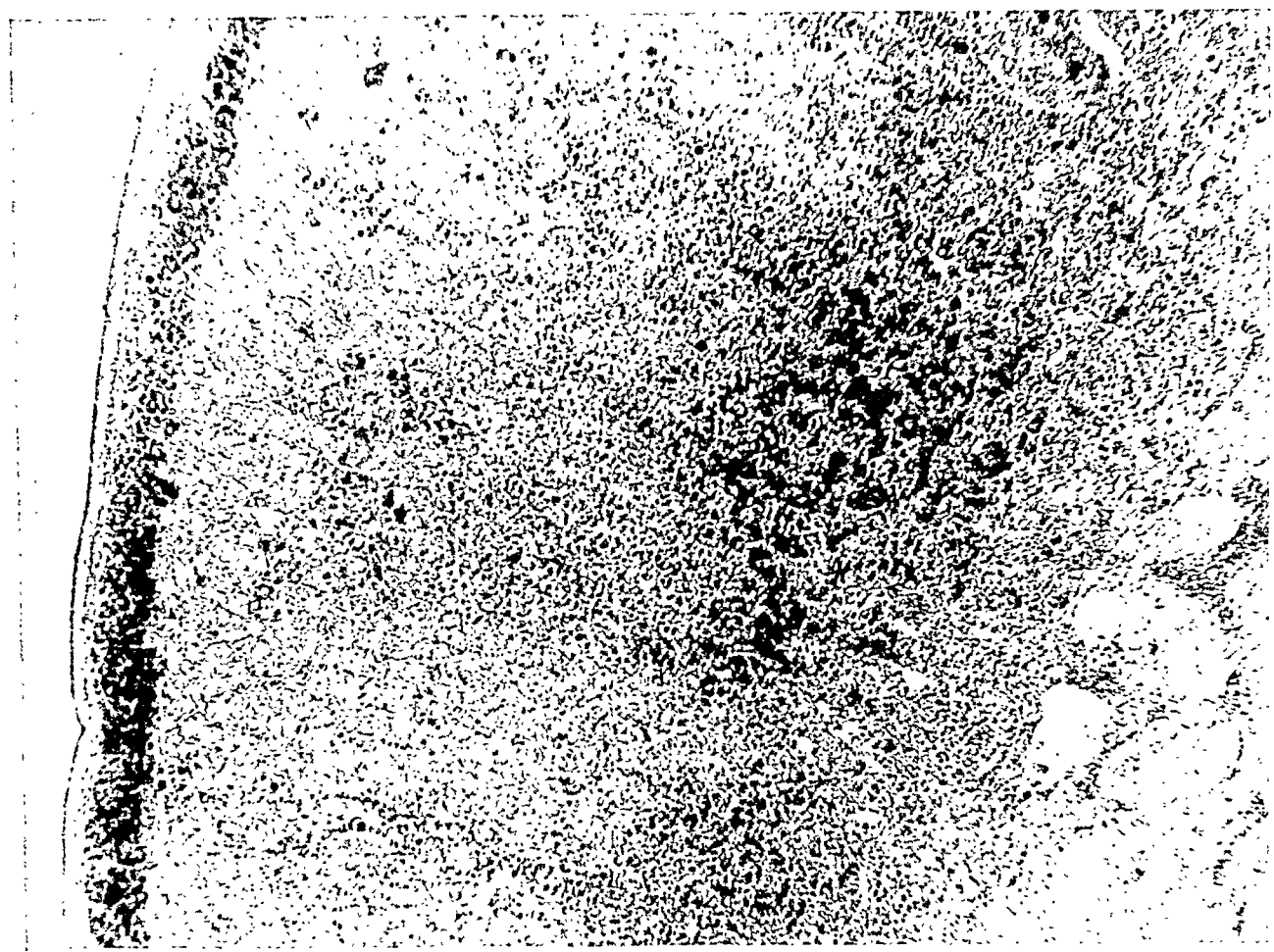
#### PLATE 41

FIG. 1. Adrenal gland from a normal animal, showing the normal distribution of lipids. Sudan III stain.  $\times 135$ .

FIG. 2. Adrenal gland from dehydrated animal 10, showing depletion of lipids in the inner zones, especially the zona fasciculata and outer portion of the zona reticularis. Sudan III stain.  $\times 135$ .



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2





# PLASMA CELL HYPERPLASIA AND HYPERGLOBULINEMIA IN TRICHINOSIS

## THE DURATION OF LARVIPOSITION \*

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Because of certain clinical manifestations, a patient at the National Naval Medical Center was thought to have multiple myeloma, and it was not until an autopsy was performed that the true nature of the disease process—trichinosis—was ascertained. It is believed that this is the first recorded instance in which plasma cell hyperplasia and hyperglobulinemia have been observed in a patient infected with *Trichinella spiralis*. The question naturally arises as to whether the plasma cells were a manifestation of an inflammatory response to the nematode, or whether an incipient form of multiple myeloma was developing simultaneously. For reasons later to be discussed, a neoplastic process, in all probability, is untenable.

Of perhaps more significance, especially from a clinical viewpoint, is the maximal duration of the intestinal phase in the human host. It is obvious that so long as gravid females are present in the intestinal tract, larvae will continue to migrate through the tissues, thus precluding recovery. Adult worms in the intestine have been reported in only a few cases,<sup>1-7</sup> and in some, microscopic confirmation has been lacking. Six weeks is the time factor generally stated for the duration of larvipositing by adult female trichinae, but this figure has been determined principally from studies in animals, particularly guinea-pigs.<sup>8</sup>

Conclusions drawn from animal experiments generally are not applicable to human trichinosis since there are definite species differences which modify the duration of the disease. In addition, the number of cysts ingested, the number of previous exposures, and the immunity acquired by these exposures considerably alter the host-parasite relationship, especially with respect to the viability of trichinae. Horlick and Becknell<sup>6</sup> reported a case of trichinosis, the duration of the infection of which was 30 days; adult worms in the intestine were confirmed by microscopic examination. Recently, Stryker<sup>9</sup> presented a case with, up to that time, the longest period of persistence of adult trichinae in the human intestine. The adult trichinae, including gravid females, were demonstrated in the intestine 54 days after ingestion of infected pork. This author emphasized, and the case to be presented reaffirms, the im-

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portance of the continued release of larvae over even possibly greater periods of time in the therapeutic management of trichinosis. In the case which follows, adult trichinae, including gravid females, were observed microscopically in the mucosa of the duodenum and ileum 115 days after the onset of symptoms.

### REPORT OF CASE

N. P., a 61-year-old veteran of World War I, was admitted to the orthopedic service of the U. S. Naval Hospital on March 4, 1946, with the chief complaint of "pain in the back." He had been in good health until 4 weeks previously when he developed a moderately severe nonproductive cough which lasted 5 days. He denied gastro-intestinal complaints. About 3 weeks before admission, he experienced sharp, persistent pain and spasm in the muscles of the back, first on the right side and then on the left. The pain, occasionally relieved by lying flat in bed, was aggravated by palpation and active motion. Pain or tightness in other muscle groups was denied. At no time had he noted skin rashes, hemorrhages, or periorbital edema. The patient lived alone. A history of ingestion of poorly cooked or raw pork was not obtained.

On admission, physical examination revealed a rather poorly nourished white male, weighing 140 pounds. He was well oriented and complained bitterly of back pain. The skin was flabby but no rashes, eruptions, discolorations, or scars were present. There was no edema, pallor, cyanosis, or dyspnea. The extra-ocular movements were normally performed, and elicited no pain. The heart was normal with respect to size, rate, and rhythm. No murmurs were heard. The blood pressure measured 130/70 mm. of Hg. The lungs were clear to percussion and auscultation. The general gait and posture supported the significant findings of an intermittent painful spasm of low back muscles, and local tenderness. True sciatic radiation was absent. Roentgenograms of the chest, spine, pelvic girdle, skull, and long bones revealed only moderate diffuse osteoporosis compatible with the patient's age and state of nutrition.

The admission laboratory examinations gave the following results: Kahn test, negative; urinalysis, unremarkable except for 10 mg. per cent of albumin; sedimentation rate, 30 mm. in 1 hour with a maximum fall of 17 mm. in 5 minutes; erythrocyte count, 3,100,000 per cmm.; hemoglobin, 9.5 gm. (66 per cent); leukocyte count, 6,000 per cmm. The differential count revealed 52 per cent neutrophilic polymorphonuclear leukocytes, 21 per cent lymphocytes, 12 per cent monocytes, and 15 per cent eosinophils. The erythrocytes showed marked hypochromia and slight anisocytosis. Because of the rather striking rouleaux formation, repeated tests for Bence-Jones protein were performed, but none was found.

On March 13, 1946, application of a spinal brace made the patient more comfortable. He had been receiving physiotherapy, and by March 28th his condition was considerably improved. This improvement continued until April 15th, when the patient complained of sharp pain in both hips with radiation down the posterior aspect of both thighs. The pain persisted, and on April 22nd he received a bilateral hip cuff injection of 2 per cent procaine, but obtained no relief. On April 25th, the total serum proteins were 12.8 gm. per cent, with 5.4 gm. per cent of albumin, and 7.6 gm. per cent of globulin.

The patient continued to suffer back pain, and on May 6th, the 63rd hospital day, he was transferred to the medical service for further investigation of his anemia and hyperglobulinemia. At that time his temperature, pulse, and respirations were normal and had been so since admission. The sedimentation rate was markedly elevated, and the urine continued to show a small amount of albumin. Occult blood was observed repeatedly in the stools. Hyperglobulinemia and hypochromic anemia

persisted, but despite repeated differential blood counts, the 15 per cent eosinophilia present on admission was not observed again. The values of all other laboratory tests, except those mentioned, were within normal limits prior to the terminal episode.

All diagnostic studies were interrupted during the patient's third day on the medical service, May 8th, by the sudden development of a severe pulmonary infection. Roentgenograms of the chest confirmed the clinical diagnosis of consolidation of the right lung with pleural effusion. The patient was treated with penicillin, oxygen, whole blood transfusions, and intravenous fluids. Despite this, his course was progressively and rapidly downhill. Two days before death, he became lethargic and stuporous. Coma developed, and he expired on May 30, 1946, the 87th hospital day.

### *Gross Autopsy Findings*

Necropsy was performed 7 hours after death. The body was that of a well developed but emaciated 61-year-old white male. The subcutaneous fat was brilliant orange, and in some areas serous atrophy was pronounced. The musculature throughout the body showed marked loss of turgor. It was muddy brown, and some muscle groups, particularly the pectoralis, had the consistency of thick putty. Myriads of oval, friable, yellowish white, encysted trichinae, which measured approximately 0.5 mm. in length, were distributed throughout all muscle groups examined, including those of the head, neck, extremities, and the extra-ocular, pectoralis, trapezius, intercostals, diaphragm, and psoas muscles.

The heart weighed 300 gm. Serous atrophy of the epicardial fat was marked. The cardiac musculature was of normal thickness, but it was muddy brown and decidedly flabby. No encysted trichinae were observed, and there was no evidence of fibrosis or infarction. The coronary and great vessels showed moderate atheromatous changes.

The left lung weighed 600 gm.; the right, 680 gm. The lower lobe of the left lung and the middle and lower lobes of the right lung were voluminous, and on section, yellowish gray, elevated, granular areas of consolidation with numerous abscesses and areas of cavitation were found. The bronchi and bronchioles were congested and contained much mucopurulent material. The tracheobronchial lymph nodes were soft and enlarged.

The mucosa of the entire small bowel was markedly congested and edematous. An occasional punctate area of hemorrhage was found, but no areas of erosion or ulceration could be demonstrated. No trichinae were seen. The remainder of the gastro-intestinal tract, together with the spleen, pancreas, liver, gallbladder, biliary tract, and adrenal glands, showed no significant abnormalities.

The left kidney weighed 180 gm.; the right, 190 gm. The capsules stripped readily, revealing smooth reddish brown surfaces. At the

superior pole of the right kidney, several well circumscribed abscesses, 3 to 4 mm. in diameter, were present. The bladder was distended and the mucosa was decidedly hyperemic. Numerous small, confluent abscesses ramified through all lobes of the prostate gland. Many segments of the periprostatic veins contained ante-mortem thrombi. The urethra, seminal vesicles, and testes were not remarkable.

There was generalized osteoporosis of the skeletal system. The vertebral, costal, and femoral marrows were soft and reddish-gray, and certain areas suggested increased cellularity. No obvious neoplastic tissue was found, however, and no focal areas of bone destruction could be demonstrated.

There was mild generalized lymphadenopathy, most marked in the mesenteric nodes. Nowhere was the architecture distorted.

The brain weighed 1420 gm. On removing the intact dura, the leptomeninges were found to be covered with a rather thick, yellow, fibrino-purulent exudate which extended over the entire convexity of the cerebral hemispheres and the greater portion of the cerebellar hemispheres. The base of the brain was bathed in pools of pus, and the exudate was particularly conspicuous along the cerebral vessels and about the longitudinal fissure. There was flattening of the convolutions in the frontal and parietal areas. The lateral ventricles contained slightly cloudy fluid, but there was no displacement or dilatation of the ventricular system. The basal arteries showed moderate arteriosclerotic changes.

The thyroid, parathyroid, and pituitary glands were virtually normal.

#### *Post-Mortem Laboratory Studies*

Chemical examination of the blood: Nonprotein nitrogen, 282 mg. per cent; blood urea nitrogen, 192 mg. per cent; creatinine, 5 mg. per cent; ammonia, trace. Bacterial cultures of the heart's blood, lung, and brain: Luxuriant growth of *Staphylococcus aureus*.

#### *Microscopic Examination*

The muscle fibers of the heart were normal on microscopic examination. Scattered diffusely throughout the interstitial tissue of the myocardium were small nests of lymphocytes and mononuclear cells with occasional neutrophils and plasma cells. The inflammatory reaction, although minimal, was most conspicuous about capillaries. Occasional small areas of scarring were present. An occasional capillary was thrombosed. No larval or encysted forms of *T. spiralis* were found.

The pleural surfaces of the lungs were covered with thick masses of fibrin in which numerous areas of hemorrhage were present. The alve-

olar spaces were choked with acute inflammatory exudate. Areas of necrosis and abscess formation were present. The blood vessels were engorged and some were thrombosed. The bronchioles were filled with inflammatory exudate. Eosinophils were not especially numerous, but there were many focal clusters of plasma cells in the interstitial tissue. No trichinal larvae were found.

The architecture of the spleen was well preserved and the malpighian bodies were not hyperplastic. Sections from different parts of the organ revealed a diffuse infiltration of mature plasma cells throughout the red pulp. Frequently, these cells were found in clumps of 3 to 6, but nowhere were they replacing the normal cellular elements of the spleen. The reticulohistiocytic cells were increased in number, and many were swollen and granular. Both intracellular and extracellular deposits of hemosiderin were present. The sinusoids were distended with blood.

The entire gastro-intestinal tract was congested, and there was rather marked edema of the mucosa and submucosa, particularly in the small bowel. The lymphatic spaces were moderately dilated. There was considerable epithelial desquamation, but no ulcers were observed. In the lamina propria and the submucosa there were numerous plasma cells and lymphocytes, and occasional neutrophilic polymorphonuclear leukocytes. Eosinophils, although present, were not numerous. In many sections of the duodenum and upper jejunum, viable adult female forms of *T. spiralis*, some of them gravid, were present deep in the crypts of Lieberkühn and in Brunner's glands. The organisms were confined entirely to the mucosal and submucosal layers. None were found in the lymphatics, and there was no marked inflammatory response in the immediate vicinity of the parasites. The esophagus, stomach, and colon, except for minimal congestion, were unremarkable.

The renal tubules in some areas were moderately dilated. The tubular epithelium was intact, but many of the cells were swollen and granular. Clusters of acute inflammatory cells were observed in the tubules, and the interstitial tissue of the cortex and medulla, in some regions, was replaced by small confluent abscesses. Mild subacute inflammation of the mucosa and submucosa of the urinary bladder was present. Numerous confluent abscesses were scattered throughout the prostatic tissue. There was focal hyperplasia of glandular epithelium and fibromuscular stroma.

Sections of cervical and abdominal lymph nodes revealed distention of the lymphatic spaces with pink-staining protein material. Scattered indiscriminately throughout all portions of the nodes were numerous mature, normal-appearing plasma cells. Many were found singly, but

the majority occurred in clusters of 2 to 4 cells. When present in clusters, they appeared to be intimately associated with the reticulohistiocytic cells. As in the spleen and bone marrow, the plasma cells were arranged so haphazardly throughout the tissue as to suggest a diffuse hyperplastic process—decidedly not a neoplastic one. The reticulohistiocytic cells were increased in number, and frequently, swollen, granular forms were observed. The follicles were not enlarged, nor was there any evidence of a transition from lymphocytes to plasma cells.

Multiple sections from costal and vertebral marrows revealed rather marked plasmacytic hyperplasia. The plasma cells were intermingled with the other blood cells. Myeloblasts, myelocytes, and megakaryocytes were present in the usual distribution. There was a moderate increase in the number of normoblasts. Occasional clusters of from 5 to 8 plasma cells were observed, but by and large, the infiltration was diffuse, and nowhere did the cells assume neoplastic proportions. The bony trabeculae revealed no areas of destruction.

In Giemsa and phosphotungstic acid-hematoxylin preparations, the majority of cell types in the bone marrow were identifiable. With few exceptions, the plasma cells were of uniform diameter and were readily differentiated by their characteristic cytoplasmic and nuclear structure. The reticulohistiocytic cells were increased and frequently showed granularity and swelling. Although no attempt was made to ascertain the origin of the plasma cells, it was observed that in the spleen and lymph nodes as well as in the bone marrow plasma cells were generally most numerous in areas where reticulohistiocytic cells predominated. Furthermore, certain cytoplasmic and nuclear changes in many of the reticulohistiocytic cells suggested that the plasma cells took their origin from these cells.

Multiple sections from various skeletal muscles throughout the body revealed a large number of trichinal larvae, some nonencysted, but most in stages of encystment. Beginning capsule formation was observed in a few instances. The recently encysted larvae were mixed with older forms, the capsules of which were homogeneous and hyalinized. Partially calcified cysts were numerous. Calcification began at the poles of the lemon-shaped cysts, usually as fine, blue-black granules. Complete calcification was observed rarely. The local inflammatory response was minimal or absent except about the nonencysted larvae. Here, a foreign body reaction with moderate acute to subchronic inflammation was noted.

The leptomeninges over the cortical surface and at the base of the brain exhibited an extensive acute inflammatory reaction with abscess

formation. The subcortical brain tissue revealed small areas of hemorrhage and minute focal clusters of inflammatory cells and macrophages about the capillaries. No granulomatous nodules were observed and no trichinal larvae were found.

Sections of the liver, pancreas, adrenal, thyroid, parathyroid, and pituitary glands were unremarkable.

*Final Diagnosis.* Disseminated trichinosis; *Staph. aureus* septicemia; confluent bronchopneumonia with abscess formation; acute suppurative meningitis; benign prostatic hyperplasia with acute suppurative prostatitis; acute focal pyelonephritis; subacute myocarditis; diffuse plasma cell hyperplasia; hyperglobulinemia; hypochromic, microcytic anemia; diffuse osteoporosis; uremia, terminal.

### DISCUSSION

The case presented is that of an infection with *T. spiralis*, complicated terminally by *Staph. aureus* septicemia, pyemia, and uremia. The striking rouleaux formation, hypochromic anemia, and hyperglobulinemia, together with other suggestive clinical manifestations, supported the tentative clinical diagnosis of multiple myeloma. In addition to the plasma cell response and the sequelae thereof, certain clinical and pathologic features of trichinosis as related to the present case require comment.

At autopsy a small piece of grossly infected pectoralis major muscle was crushed between two glass slides and examined microscopically. Nonencysted trichinae exhibited their rather characteristic spiral motion, and excystment of some larvae with subsequent curling and uncurling was observed. A quantitative estimation of the infection was ascertained with the use of the trichinoscope. Since it is sometimes difficult to detect noncalcified encysted and nonencysted trichinae because of their transparency, the skeletal muscle was fixed in 10 per cent formalin. The fixation increases the opacity of the parasites and their capsules, and thereby greatly facilitates their detection. The formalin-fixed tissue was then dried, and 1 gm. portions were teased apart and placed between the glass plates of the trichinoscope. The results of two of the counts are shown in Table I.

If one disregards those trichinae in which the capsules were completely calcified, then the degree of infection, as determined by Hall and Collins,<sup>10</sup> may be considered to be moderate to heavy. This in itself, however, means little with regard to possible fatal outcome or even clinical manifestations. Gould,<sup>11</sup> for example, has found trichinous infection of 101 to 1000 larvae per gm. of muscle at autopsy in each of



11 persons who died from diseases other than trichinosis. Furthermore, in none of these 11 persons was there a clinical history of trichinosis. It has been repeatedly observed that many variables such as size and age of the patient, nutritional status, and the presence of other concomitant morbid processes in the body may ultimately determine the subsequent sequence of events and the end result. Nevertheless, the fundamental disease process in this case may be regarded, justifiably, as one of trichinous infection.

In regard to the biologic history of *T. spiralis* as it pertains to this case, it should be emphasized that adult female worms, some of them gravid, were found in the mucosa of the duodenum and jejunum. It is generally believed that following copulation, the male intestinal trichina

TABLE I  
*Incidence of Larval Forms in Skeletal Muscle*

Larval form	Diaphragm	Psoas
Nonencysted	8	5
Encysted	76	25
Beginning calcification of capsule	58	26
Complete calcification of capsule	2	5
Total	144 per gm.	61 per gm.

dies and is digested, and that following the birth of the larvae, the female intestinal trichina likewise dies and is digested.<sup>12</sup> According to Cameron as quoted by Faust,<sup>13</sup> the young females are fertilized on about the 3rd day after ingestion of infected meat, and begin to larviposit on the 5th day. Larvipositing decreases by the 14th day. Of more significance, however, is the fact that the maximum life span of the mother worms in the intestinal wall is not known with certainty. In the case presented, if we date the length of the disease from the onset of the respiratory symptoms, then viable adult female intestinal trichinae were present for 115 days. If we accept the general observation that respiratory symptoms develop 2 to 6 days following the ingestion of infected meat, then the adult female trichinae were present for approximately 120 days. Assuming that the severe nonproductive cough (4 weeks prior to admission) was totally unrelated to the trichinous infection, the patient was, nevertheless, in the hospital under close observation for a total of 87 days. There is, of course, the possibility of reinfection. If so, the source might have been the hospital food, a fact which has not been overlooked. Meat was carefully inspected, and nothing was found to incriminate it as a possible source. Furthermore, no cases of clinical trichinosis were

found in the hospital personnel or in the other 1100 patients who ate similar food at all meals. The patient was too ill to subsist outside the hospital, and as far as could be determined, no food was sent to him from the outside. It would seem most probable, therefore, that the infection was acquired before entering the hospital, in all probability approximately 5 weeks before admission. The presence of nonencysted motile larvae in muscle also proves that larvipositing can occur for at least 4 months. Since the overwhelming majority of larvae counted were either encapsulated or showed beginning calcification of the capsules, and since encapsulation begins about the 21st day of the infection, and beginning calcification of the capsules at about 6 months,<sup>13</sup> it would seem quite possible that the patient consumed infected meat on more than one occasion. Furthermore, it would appear that the greatest number of trichinae were consumed about 5 months apart. And yet in view of finding adult female intestinal trichinae and nonencysted larvae in muscle 4 months from the onset of the illness, it would appear equally possible that the infection could have been acquired after just one exposure. The presence of a few larvae with complete capsular calcification, a process which is believed to require about 1 year at least, indicates that these must have been derived from a previous infection.

In an attempt to integrate the clinical findings as related to trichinosis, it will be noted from the history that the onset of illness 4 weeks prior to admission was characterized by a moderately severe, nonproductive cough which lasted 5 days. This symptom, although usually not a serious one, is present in approximately 30 per cent of cases and frequently marks the onset.<sup>14</sup> The cough is generally attributed to the passage of larvae through the pulmonary capillaries in their transit from the intestinal mucosa to the peripheral circulation.

One week following the onset of the cough, pain in the muscles of the back made its appearance. It is not unusual in trichinosis for pain to be limited to one or two muscle groups such as the back and thigh,<sup>15</sup> and it occurred at a time when the migration of larvae to skeletal muscle might be anticipated. Of the muscles of the back, the lumbar group are apt to be the most heavily seeded.<sup>16</sup> The pain gradually increased in severity, and although aggravated by both active and passive motion, it was present also at rest—findings quite in keeping with the character of muscle pain in trichinosis.

The absence of certain clinical manifestations which are so commonly a part of trichinosis is of considerable interest. There were no abdominal symptoms. Ocular movements elicited no pain, and there was no edema, generalized or local. Fever was not present terminally, nor were

there chills, sweats, or malaise. Hypochromic anemia was moderate, and eosinophilia (15 per cent) was found on the admission count only. The absence of these manifestations, however, does not preclude the diagnosis of trichinosis.

Although myocarditis constitutes the chief danger in trichinosis, and subsequent congestive heart failure is the most common cause of death, the subacute myocarditis in this case was minimal and probably contributed only slightly, if at all, to the patient's death. Septicemia is considered a rather rare complication of trichinosis,<sup>17</sup> but *Staph. aureus* septicemia, pneumonia, meningitis, and pyelonephritis were the terminal events in this case.

### *The Plasmacytic Reaction*

The presence of numerous plasma cells in the bone marrow, lymph nodes, and spleen was a thought-provoking finding. Was the plasmacytic response an unusual manifestation of trichinosis, or was a concomitant multiple myeloma or myelomatosis developing? The indiscriminate distribution of typical mature plasma cells among the normal tissue cells, rather than the presence of obvious aggregates of these cells, is evidence in favor of the former. The absence of discrete skeletal defects, tumor formation, and other pertinent clinical manifestations frequently observed in multiple myeloma argues against this diagnosis. Yet when one considers the clinical and pathologic variations that may occur in the group of myelomatous diseases, particularly in the early stages, there remains considerable doubt concerning the true nature of the plasma cells in this case.

A variant of the plasma cell tumors, the so-called generalized or diffuse myelomatosis, has been described particularly by the French and Scandinavian writers.<sup>18-21</sup> It is a common experience to find that with multiple discrete plasma cell tumors there is, to a greater or lesser extent, a diffuse distribution. But a diffuse malignant plasma cell invasion of the skeleton and some viscera without evidence of focal neoplastic masses must be quite rare. Geschickter<sup>22</sup> has yet to observe such a case. Marchal and Mallet,<sup>20</sup> on the other hand, have reported a case in which there was extensive diffuse plasma cell invasion of the skeletal system. The roentgenograms of the skeleton revealed diffuse radiolucency without cysts, erosions, or punched-out areas. Their patient exhibited no tumors nor Bence-Jones protein, but did maintain hyperglobulinemia and moderate hypochromic anemia. These authors were convinced that their case was one of plasma cell myeloma. Their findings are comparable to those in the present case in respect to plasmacytosis. However,

some of the reported cases of diffuse myelomatosis are of doubtful authenticity as to being fundamentally neoplastic. Tuberculosis, as well as certain other chronic diseases in which there is frequently a proliferation of plasma cells and hyperglobulinemia, cannot be excluded entirely.<sup>19</sup> Furthermore, there is often considerable doubt as to the significance of the bone changes on roentgenographic examination. In the vast majority of reported cases of diffuse myelomatosis, the patients were in an age group in which the changes of senile osteoporosis might be expected to occur.

A detailed study of the structure of the plasma cells in this case was of no practical value in determining whether the cells were fundamentally neoplastic. The majority appeared mature and similar in all respects to the plasma cells commonly found in chronic inflammatory lesions. Even in the group of extramedullary plasmacytomas, it is often impossible to determine whether an individual tumor is benign or malignant. As Hellwig,<sup>23</sup> in his general review of the subject, aptly pointed out, the microscopic appearance of the plasma cell cannot be relied on for prognosis.

When we consider the more common plasmacytic response which results from certain injurious agents, we find many diseases in which this cell is an outstanding feature of the cellular reaction. Many chronic and granulomatous infections such as tuberculosis, syphilis, sarcoid, leprosy, lymphogranuloma inguinale, bronchiectasis, pyonephrosis, rheumatoid arthritis, kala-azar, filariasis, and schistosomiasis, are characterized by proliferation of plasma cells during some stage of their course. In none of these diseases is there anything particularly remarkable about the morphologic characteristics of these cells. Quantitatively, they vary with the etiologic agent, the severity of the infection, and the general resistance of the tissues. It is common knowledge, too, that hyperglobulinemia frequently is associated with the above entities, at least during some phase of the disease process.<sup>24-26</sup> The hyperglobulinemia is seldom, if ever, as marked as with malignant myelomas, but neither is the plasma cell response so extensive or diffuse. Many of the above diseases are characterized by involvement of the reticulohistiocytic system as well as by proliferation of plasma cells. This fact supports the theory that some serum protein is formed by the plasma cells and the cells of the reticulohistiocytic system,<sup>24,26-30</sup> thus stressing the functional relationship between the two types. The findings in the case presented are in accord with the above statement in this regard.

So far as I am aware, there has been no other report of a plasma cell response with associated hyperglobulinemia in a case of trichinosis.

Thus, although a definitive statement concerning the fundamental nature of the plasma cell response in the present case cannot be made, all factors considered, it appears highly probable that the plasma cells constitute a primary cellular response to trichinosis.

### SUMMARY

Viable adult trichinae, including gravid females, were observed microscopically in the small intestine of a fatal case of human trichinosis 115 days after the onset of symptoms. The patient was continuously hospitalized and under close observation for 87 days. Reinfection, although remotely possible, was considered to be adequately excluded. The period of persistence of living adult trichinae in the human intestine is the longest thus far recorded. The continued release of larvae over relatively long periods of time is of considerable significance from a clinical, prognostic, and therapeutic point of view. The degree of infection as determined by counts with the trichinoscope was considered moderate to heavy.

The diffuse plasmacytosis with associated hyperglobulinemia is considered to constitute an unusual, if not unique, cellular response to trichinosis.

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[ Illustrations follow ]

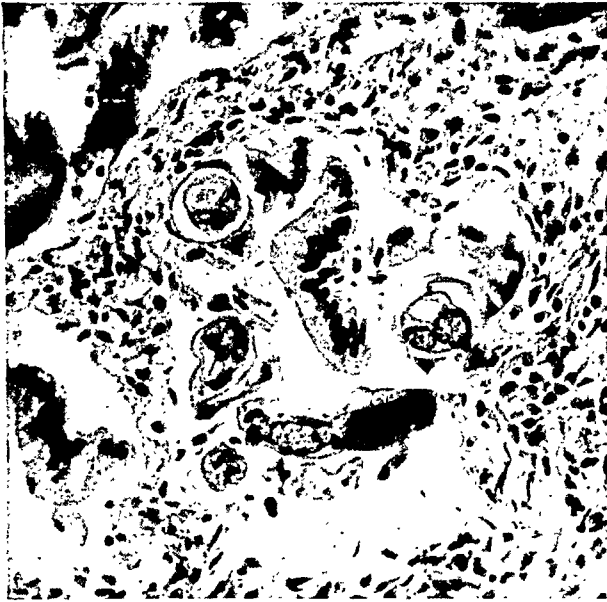
## DESCRIPTION OF PLATE

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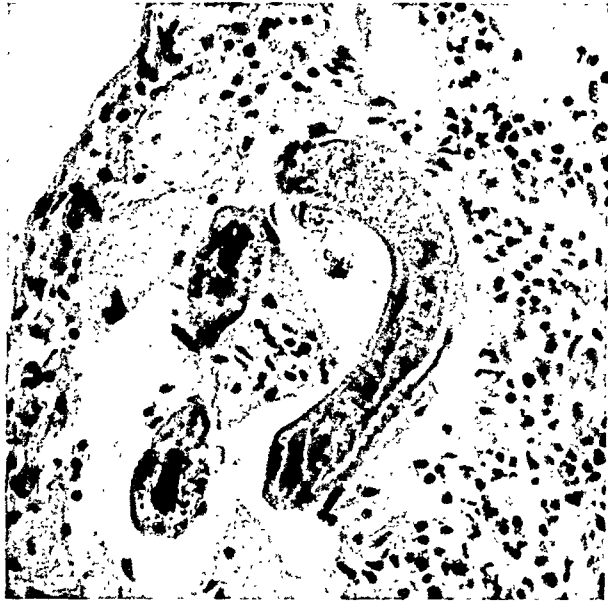
### PLATE 42

- FIG. 1. Adult trichina in mucosa of jejunum.  $\times 100$ .
- FIG. 2. Nonencysted larva in pectoralis major muscle.  $\times 100$ .
- FIG. 3. Encysted larva in pectoralis major muscle. Of note are the muscle nuclei within a cyst.  $\times 100$ .
- FIG. 4. Encysted larva in pectoralis major muscle, showing partial calcification.  $\times 100$ .
- FIG. 5. Plasma cell infiltration in vertebral bone marrow.  $\times 200$ .
- FIG. 6. Plasma cell infiltration in lymph node.  $\times 400$ .

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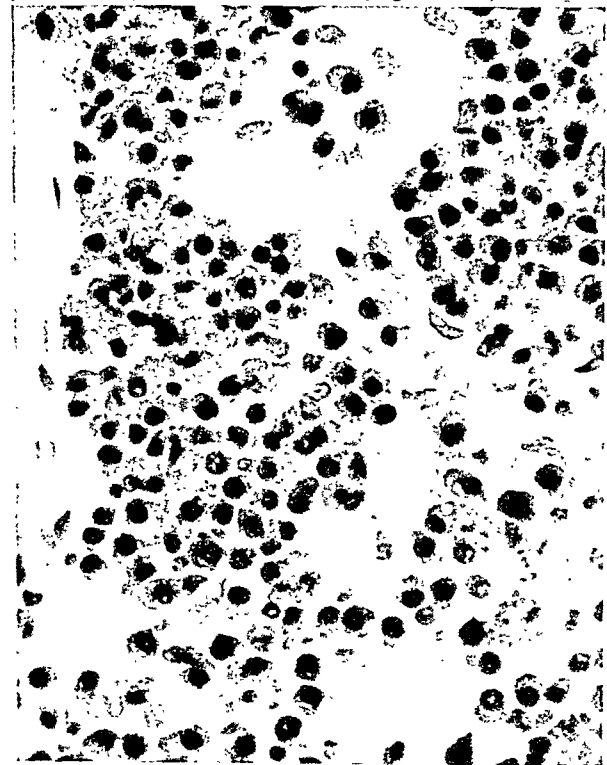
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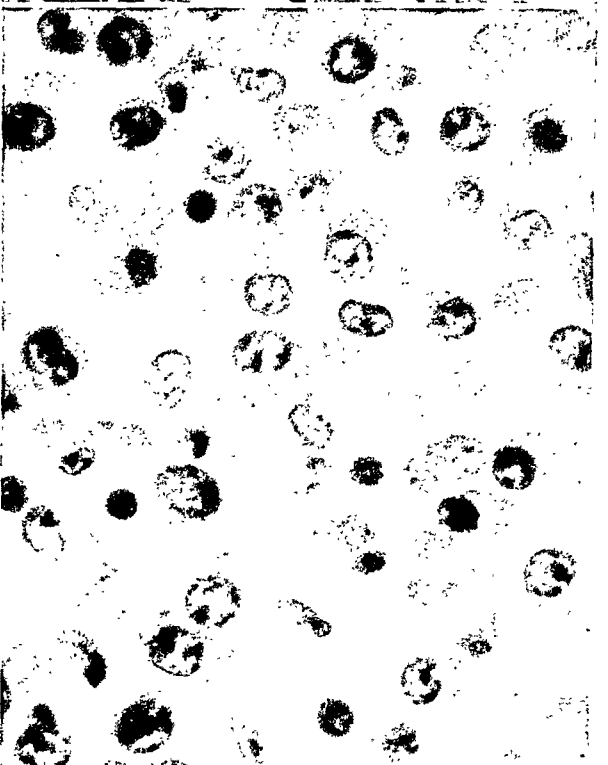
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## DIVERTICULA IN THE TERMINAL PORTION OF THE COMMON BILE DUCT \*

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Standard textbooks describe the termination of the biliary tract almost exactly as it was done by Vater,<sup>1</sup> Opie,<sup>2</sup> Oddi,<sup>3</sup> and others<sup>4</sup> prior to 1905. Recent studies by Dardinski,<sup>5</sup> Michels,<sup>6</sup> and Schwegler and Boyden<sup>7</sup> have not been included in the standard descriptions of the anatomy of the biliary tract. Surgical procedures on the common duct have increased in scope and incidence; hence there is a stimulus for more specific information regarding its termination. My studies of fresh operative and autopsy specimens have been supplemented by injection of gelatin, dyes, and radio-opaque materials, as well as by detailed dissections in formalinized specimens. An abnormality found during investigations on the terminal portion, or *pars intestinalis*,<sup>4</sup> of the common bile duct is reported.

Four diverticula of the terminal or intrapapillary† portion of the common bile duct were observed in a series of 70 anatomic dissections. A fifth specimen in this series contained a choledocho-pancreatic duct fistula. These morphologic variations have not been reported previously.

### METHOD

The duodenum, head of the pancreas, the hepatic pedicle, and adjacent tissues were removed at autopsy. In certain specimens air was injected into the duodenum after clamps had been applied to both ends. In a few specimens, diodrast or lipiodol was injected into the common bile and pancreatic ducts and radiographs were taken.

Specimens were preserved in a 4 per cent solution of formalin for 48 to 72 hours prior to complete dissection. The intact common bile duct was exposed throughout its extraduodenal course. Overlying pancreatic tissue was incised from the duodenum toward the duct. The proximal portion of the papilla was identified at the duodenal wall, and dissection was continued in order to expose the pancreatic duct as it emerged from the head of the pancreas. With fine-pointed scissors the lateral aspect of each duct was opened and the duodenal canal was entered through the papilla. The walls of the ducts were everted for inspection. Sections were cut for microscopic examination, particularly of the pancreas, and these were examined in consultation with Dr. Helen Ingleby.

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† The term *papilla* is used by Dardinski<sup>5</sup> and is the anatomic equivalent of the "*pars intestinalis*" of the common bile duct as described by Boyden.<sup>4</sup>

TABLE I  
*Measurements of the Extraduodenal Segment of the Common Bile Duct*

Diameter of the lumen		Thickness of wall	
Number of cases	Diameter (mm.)	Number of cases	Thickness (mm.)
3	3.2	16	0.4
5	4.0	22	0.8
9	4.8	2	1.2
1	5.2	2	1.6
1	5.6	Total 42	0.7 (Average)
13	6.4		
4	7.2		
5	9.6		
1	10.4		
2	11.9		
1	12.7		
Total 45	6.4 (Average)		

TABLE II  
*Measurements of Diameter of Extraduodenal Common Bile Duct on Cholangiograms*

Total series		Clinically normal patients	
Number of cases	Diameter (mm.)	Number of cases	Diameter (mm.)
3	4	3	4
2	5	2	5
7	6	7	6
5	7	4	7
2	8	2	8
4	9	2	9
4	10	4	10
2	11	Total 24	6.9 (Average)
5	12		
3	13		
1	14		
1	15		
4	16		
0	17		
0	18		
1	19		
0	20		
2	21		
3	22		
1	23		
Total 50	11.1 (Average)		

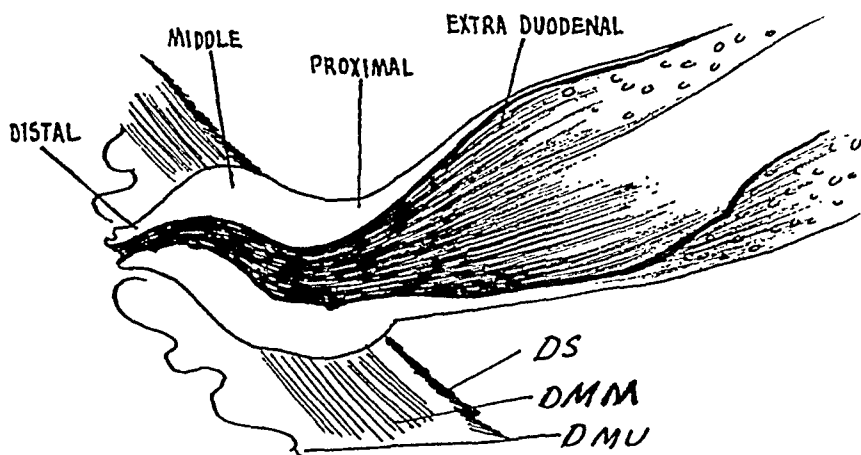
## RESULTS

A total of 70 specimens were dissected. In a series of 45 formalinized specimens, the wall of the common bile duct was found to have an average diameter of 6.4 mm., and to be 0.7 mm. thick in its extraduodenal course (Table I). The extraduodenal diameter of the common bile duct as seen on the cholangiogram averaged 6.9 mm. in the normal patient (Table II). In the gross specimen and on the cholangiogram it was observed that the normal diameter of the common bile duct was less than 1 cm. Differences in measurement between fresh and formalinized specimens were carefully studied and it was found that an error up to 5 per cent was possible.

## NORMAL STRUCTURE

On the radiograph taken after lipiodol was injected into the pancreatic duct and diodrast into the common bile duct, it was evident that each duct emptied separately into the duodenum (Figs. 2 and 3). The lumen of the common bile duct decreased in diameter as the duct crossed the duodenal wall.

Examination of the dissected specimen revealed a difference between the extraduodenal and transduodenal (*pars intestinalis*) segments of the



Text-Figure 1. Sketch indicates the major divisions of the common bile duct as (A) extraduodenal, and (B) transduodenal or *pars intestinalis*, which, in turn, is divided into proximal, middle, and distal portions from without to within the duodenum. DS = duodenal serosa; DMM = duodenal musculature; DMU = duodenal mucosa.

common bile duct (Text-Fig. 1). The extraduodenal portion had a wide lumen, a thin wall, and a smooth mucosa which was stippled by the orifices of numerous glands.<sup>5</sup> In the transduodenal segment of the common duct, the lumen became a funnel which decreased in diameter to terminate as a narrow filamentous canal. The wall of this segment was thick and the mucosal lining had many reduplications and valvules.<sup>2,5,8</sup> It was in the valvular area of the common bile duct that four diverticula and one fistula were observed.

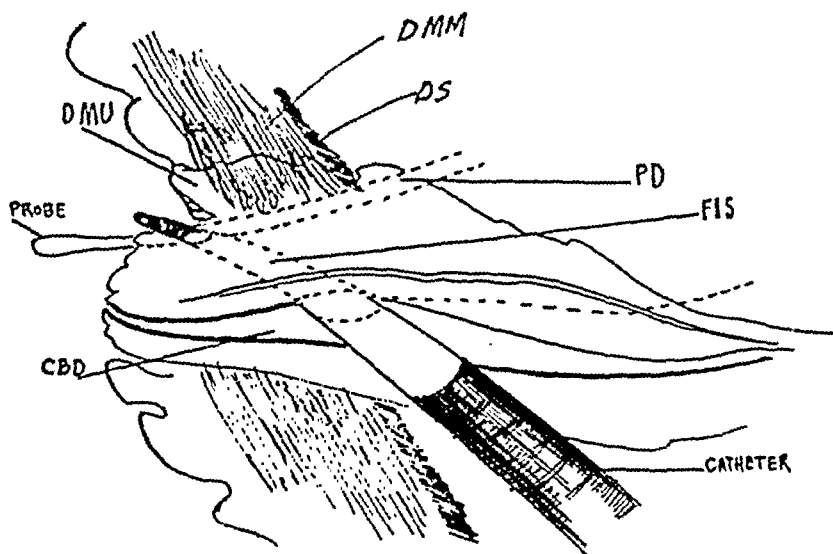
## OBSERVATIONS ON ABNORMALITIES

*Specimen 41.* In specimen 41, the diameter (6.4 mm.) and the thickness (0.8 mm.) of the wall of the extraduodenal portion of the common duct were normal. In the distal half of its pancreatic course the common bile duct was covered with pancreatic tissue. The pancreatic and common bile ducts opened through separate orifices into the duodenum. The papilla was 12.8 mm. long and its wall averaged 3.4 mm. in thickness. Four large valvules and many smaller reduplications were present

in the transduodenal segment of the common duct. Beneath a cusp of a valvule in the middle third of the intrapapillary portion of the common bile duct, a 2.0 mm. orifice admitted a probe into a tract which extended approximately 1.5 cm., to empty into the pancreatic duct 2.0 mm. from the termination of the duct in the duodenum (Text-Figure 2). The fistula was empty. There were no other abnormalities of the ducts, pancreas, or of adjacent organs.

This choledocho-pancreatic duct fistula was probably a developmental anomaly.

*Specimen 25.* The lumen (4.8 mm. in diameter) and wall (0.8 mm. in thickness) of the extraduodenal segment of the common bile duct in specimen 25 were normal in size. The common duct was intrapancreatic for one-third of its pancreatic course. The common bile and pancreatic ducts emptied through the papilla into the duodenum by separate orifices.



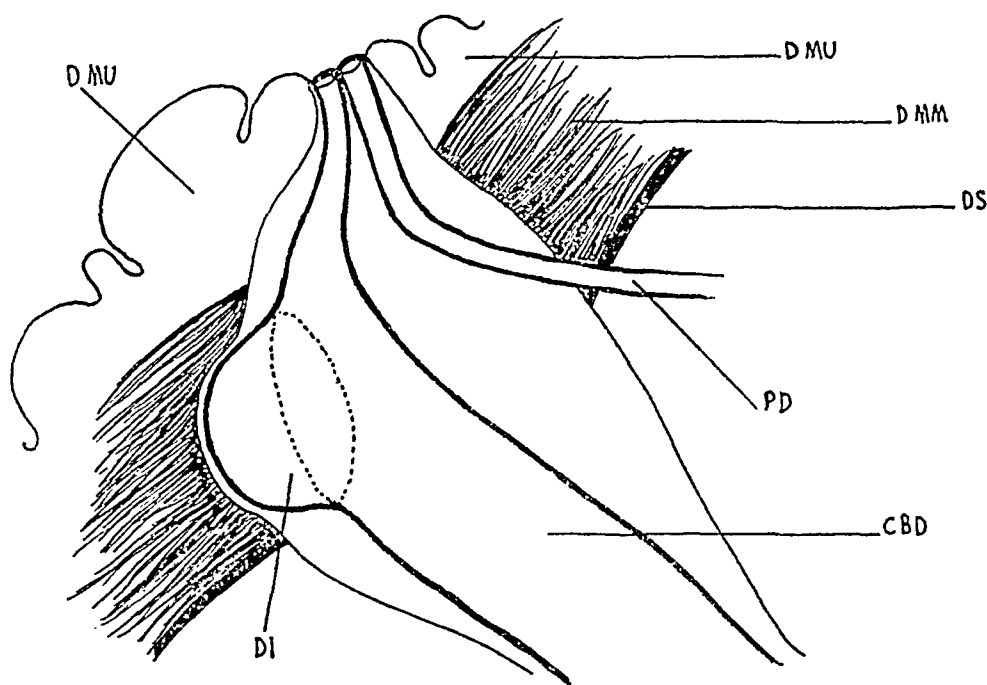
Text-Figure 2. A catheter is passed through the choledocho-pancreatic duct fistula (FIS). A probe is in the pancreatic duct (PD) emerging through the orifice into the duodenum. The orifices of the common bile and pancreatic ducts are separate. CBD = common bile duct. (Specimen 41.  $\times 5$ .)

The papilla was 18.9 mm. long and its wall was 2.4 mm. thick. There were many valvules in the papillary portion of the common duct. An area in the proximal half of the papilla was less than 1.0 mm. thick. Here a diverticulum was found emerging toward the duodenum and impinging on the duodenal wall (Text-Fig. 3). There were no calculi in the common bile duct. The pancreas was normal grossly.

*Specimen 40.* In specimen 40, the diameter (10.4 mm.) and thickness (1.2 mm.) of the wall of the common bile duct in its extraduodenal portion were slightly greater than normal. In its pancreatic portion the common bile duct was completely surrounded by pancreatic tissue. The pancreatic and common bile ducts opened through separate orifices into

the duodenum. The papilla was 14.3 mm. long and the thickest portion of its wall measured 4.0 mm. There were many valvules in the intrapapillary portion of the common bile duct. In the middle third of the papilla an orifice approximately 2.0 mm. in diameter was observed through which a diverticulum had opened. The sac was approximately 6.0 mm. long. It contained three small, black calculi and some bile-stained débris (Text-Fig. 4).

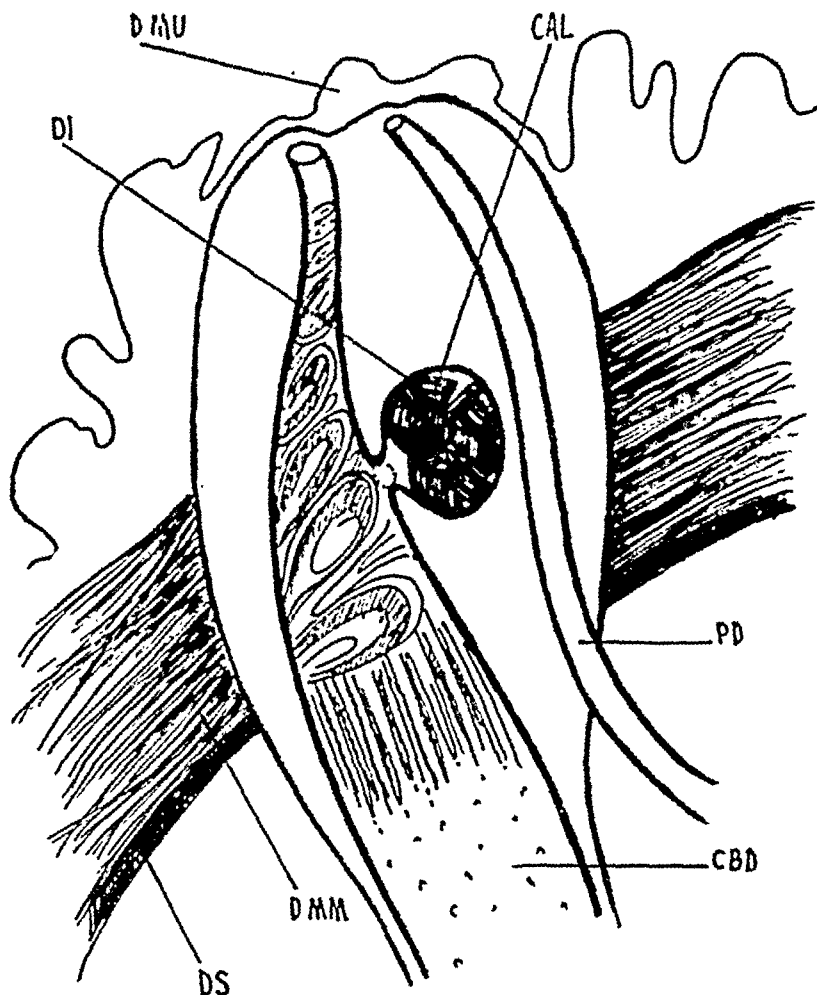
Grossly, the pancreas was firm and sclerotic. Microscopically, there was recent necrosis of the parenchyma with evidence of fatty infiltration and fat necrosis. There was severe periacinar fibrosis and minimal cellular reaction.



Text-Figure 3. A diverticulum (DI) is present in the proximal segment of the papilla. The diverticulum extends toward the duodenal wall and impinges on the serous and muscular layers. There are separate orifices for the common bile and pancreatic ducts. (Specimen 25.  $\times 5$ .)

*Specimen 63.* The patient was a white male, 63 years old, who was treated surgically on the service of Dr. Ralph Goldsmith (Jewish Hospital, Philadelphia), for a pancreatic pseudocyst and recurrent pancreatitis. The pseudocyst was drained. Several months later, cholecystectomy was performed because of cholelithiasis. The common duct was normal grossly and no calculi were found. A "T" tube was sewn into the common duct. A cholangiogram was made on the 15th post-operative day. The hepatic ducts were not dilated. There was extravasation along the course of the "T" tube. Dye entered the duodenum rapidly. There was a small irregularity in the distal portion of the common bile duct. Less than 1 cm. from the termination of the com-

mon bile duct the distal portion of the pancreatic duct was demonstrated, and both ducts had a common orifice extending through the innermost portion of the duodenal wall (Fig. 1). Massive bleeding through and around the "T" tube followed  $\frac{1}{2}$  hour after cholangiography, subsiding spontaneously. The "T" tube was removed, and the patient discharged.



Text-Figure 4. A diverticulum of the medial portion of the papilla is seen to emerge on the pancreatic aspect and to penetrate toward the pancreatic duct. CAL = calculi in the diverticulum. (Specimen 40.  $\times 5$ .)

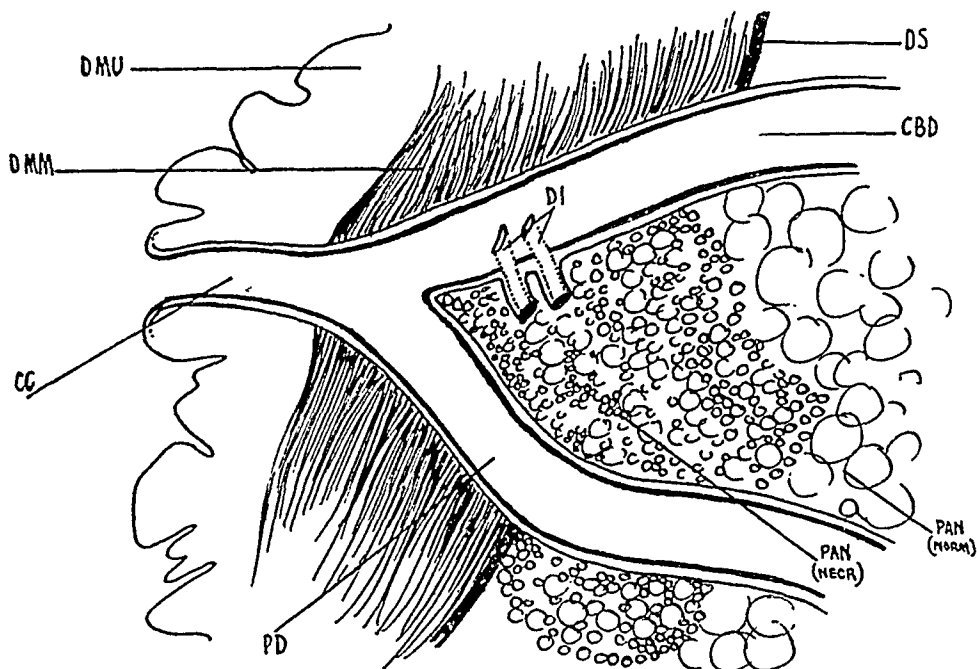
Intermittent bleeding continued. The patient was readmitted to the hospital 4 months later because of hematemesis, melena, a marked secondary anemia, jaundice, and severe hepatocellular damage. Two months later, he died of exsanguination.

The cause of death was found to be bleeding from an erosion of the right branch of the hepatic artery into the common bile duct. This was probably secondary to the action of fluid leaking from the pancreatic pseudocyst.

On post-mortem examination of the biliary tract, the lumen (5.0 mm. in diameter) and wall (0.8 mm. in thickness) of the extraduodenal common bile duct were normal. The pancreatic duct joined in a com-

mon channel with the common bile duct; this common channel was 6.0 mm. long and extended through the duodenal mucosa.

The papilla was 15.0 mm. long; its wall was less than 1.5 mm. in thickness throughout. Papillary tissues were atrophied; only flattened markings and valvular remnants were observed in the intrapapillary segment. Calculi were not found.



Text-Figure 5. Two diverticula pass from the common bile duct to terminate within an area of pancreatic necrosis (PAN, NECR). The common channel (CC) for the common bile and pancreatic ducts is distal to the location of the diverticula (DI). (Compare Figure 1.) (Specimen 63.  $\times 5$ .)

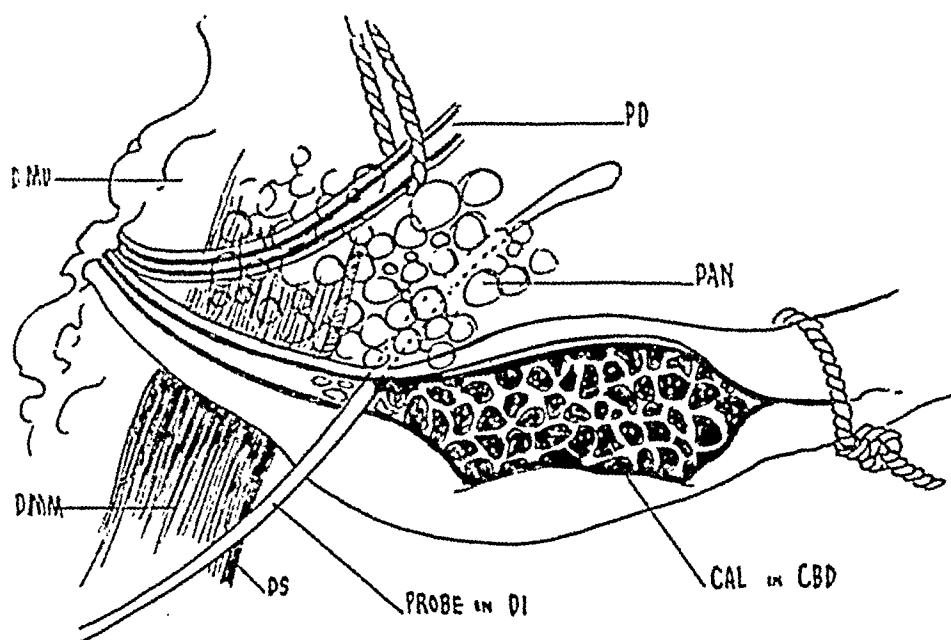
In the middle third of the intrapapillary portion of the common bile duct and proximal to the beginning of the common channel for both ducts, two oval orifices were observed, each with a diameter of 1.0 mm. (Text-Fig. 5). A probe was passed through these openings into two small sacs which extended into pancreatic tissue for 5.0 and 8.0 mm., respectively. The sacs were closed, although their walls were friable. Pancreatic tissue adjacent to the diverticula was necrotic for a radius of 2.5 cm. The body of the pancreas was sclerotic. The main pancreatic duct was dilated. In the body of the pancreas, approximately 6.0 cm. from the papilla, a sinus tract extended from the main pancreatic duct into a large cavity which had dissected the layers of the gastrohepatic omentum to form a pseudocyst.

Microscopically, the pancreas showed severe chronic pancreatitis with necrosis, diffuse inflammatory exudate, and periacinar and perilobular fibrosis.

*Specimen 36.* The diameter (6.4 mm.) and width of the wall (0.8



mm.) of the extraduodenal common bile duct of specimen 36 were normal. The duct was intrapancreatic for the distal half of its pancreatic course. The pancreatic and common bile ducts emptied separately into the duodenum. The papilla was 17.0 mm. long and its wall was 6.4 mm. thick. Valvules in the transduodenal segment of the common bile duct were thicker than is normal. On the pancreatic aspect of the papilla a thin, narrow diverticulum was found which was 8.0 mm. long and 1.5 mm. wide (Text-Fig. 6). This diverticulum contained



Text-Figure 6. The probe has entered a diverticulum originating in the proximal portion of the pars intestinalis of the common bile duct. The diverticulum ends with adjacent pancreatic tissue. (Specimen 36.  $\times 3$ .)

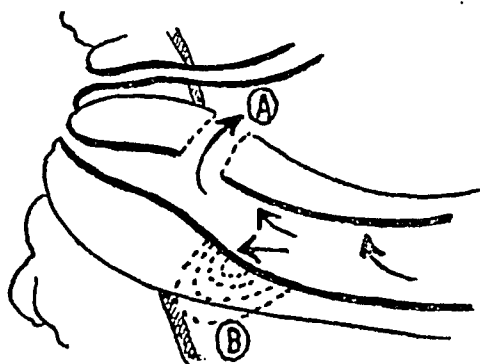
small calculi. The common bile duct contained many faceted gallstones. There was another area in the proximal portion of the papilla which, because of the extreme thinness of the wall, may have represented the primary stage in the formation of another diverticulum. Adjacent pancreatic tissue was sclerotic grossly. Microscopically, there was slight increase in perilobular fibrous tissue, with moderate edema, early degeneration, and necrosis present in a few acini.

### DISCUSSION

Obstruction to the flow of bile from the distal termination of the common bile duct may be produced by calculus, stricture, spasm, inflammation, or neoplasm. Increased intracholedochal pressure frequently occurs under such conditions, so that the normal pressure of 10 to 15 cm. of water may be increased to 60 to 75 cm. of water.<sup>9,10</sup> When increased pressure is maintained, and particularly if a calculus is present

and/or if there is a defect in the continuity of the duct, these may summate to provide an alternate route of least resistance for the passage of bile from the common duct into a diverticulum, and eventually a choledochal fistula might form.

In the distal centimeter of the common bile duct, the lumen is decreased and the wall is thicker. A stone is normally impacted proximal to these thickened tissues of the common duct papilla and the narrow filamentous portion of its lumen. There would be a tendency for a stone to erode through the thinner tissues toward the pancreas and its duct (Text-Fig. 7, A), or toward the duodenal wall (Text-Fig. 7, B) to form a choledocho-duodenal fistula. A pre-existing structural deficiency and the trauma and inflammation associated with choledocholithiasis could well be the etiologic factors in the pathogenesis of diverticula in the papillary region of the common bile duct.



Text-Figure 7. The diagram indicates potential directions taken by diverticula formed when increased intracholedochal pressure is exerted against the papilla. In "A" the diverticulum penetrates toward the pancreatic duct and in "B" toward the serosa of the duodenal wall.

Two of four reported diverticula contained biliary calculi. Pancreatitis was present in these cases. A third case with diverticula had a past history of pancreatitis and of a pancreatic pseudocyst. In that case, cholecystectomy was done for cholelithiasis. However, on final examination choledocholithiasis could not be demonstrated. In the fourth specimen of a diverticulum there was neither evidence of pancreatitis, nor were calculi found in the common duct, gallbladder, or pancreas.

Calculi in a diverticulum may be asymptomatic. The stone and diverticulum might escape observation on the postoperative cholangiogram if the orifice of the sac was occluded by the stone or by edema. On the other hand, the escape of bile or of a calculus through such a diverticulum into adjacent tissues might be manifest clinically as an episode of pancreatitis. This becomes a means for the introduction of bile into the pancreas.<sup>11-19</sup> When pancreatitis is evident, specimens of the distal segments of the common bile and pancreatic ducts should be carefully examined after preservation in formalin. Unless their existence is suspected and observation directed toward their demonstration, diverticula in the terminal portion (*pars intestinalis*) of the common bile duct can be overlooked.

## CONCLUSIONS

Abnormal channels in the terminal portion of the common bile duct were found in 7 per cent of a series of 70 anatomic dissections.

Four diverticula and one choledocho-pancreatic fistula are reported. Two diverticula contained stones. In these two specimens there was gross and microscopic evidence of pancreatitis.

One patient with diverticula of the common bile duct had a history of pancreatitis and cholelithiasis. There were no calculi demonstrated in the common duct nor in the diverticula at the time of examination of the specimen.

Diverticula of the common bile duct may be a factor in the pathogenesis of certain forms of pancreatitis.

A diverticulum in the terminal portion (*pars intestinalis*) of the common bile duct is easily overlooked. It is recommended that careful dissection of the formalinized specimen be done in cases presenting a history of pancreatitis.

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[ *Illustrations follow* ]

## DESCRIPTION OF PLATE

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### PLATE 43

FIG. 1. On this indirect cholangiogram diodrast entered the duodenum without obstruction to its flow. There is a concavity of the common hepatic and bile ducts due to pressure by a pancreatic pseudocyst. The medial convexity of the distal segment of the common bile duct terminates as a small S-shaped curve.

The last centimeter of the pancreatic duct as well as 7 mm. of a common channel for the pancreatic and common bile ducts is visualized.

FIG. 2. Diodrast injected through the catheter in the common bile duct entered the duodenum through a funnel-like lumen which terminated as a filamentous canal.

FIG. 3. Lipiodol injected through a needle into the pancreatic duct diffused through the pancreatic ductal system. It emptied into the duodenum alongside the common bile duct, but through a separate orifice.

1



2



3





# NEOPLASTIC DISEASES OF DOGS

## I. NEOPLASMS OF MELANIN-FORMING CELLS \*

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Among the papers in a recent symposium<sup>1</sup> devoted exclusively to a consideration of melanoma, no example of a neoplasm of melanin-forming cells in dogs was recorded. The scarcity of melanoma in the dog is attested by the relatively few cases in several compilations<sup>2-13</sup> of tumors in dogs found in the literature. For this reason an analysis has been made of the melanin-containing neoplasms of the skin of 31 dogs, with regard to their gross and microscopic features. These have been correlated with available information on breed, age, sex, sites of predilection, and some clinical details.

### REVIEW OF LITERATURE

In an extensive survey of the early literature of tumors in animals, Casper<sup>2</sup> found 3 instances of melanoma in dogs, the first observed by Kitt<sup>3</sup> in the neck, the second by Bruckmüller<sup>4</sup> at the base of the brain as small tumors, and the third by Bournay<sup>5</sup> in the mouth, shoulder, heart, and lungs as multiple sarcoma, partly melanotic and partly telangiectatic. McFadyean<sup>6</sup> noted a "melanotic carcinoma" of the skin of the back among 23 cases of carcinoma in dogs. Of 26 cases of canine sarcoma, Murray<sup>7</sup> found "melanotic sarcoma" of a hind leg in 2 dogs. Although he had a wide personal experience with tumors in dogs, Joest<sup>8</sup> recorded but one instance of melanoma, a "melanosarcoma" of the nose with metastases in the lungs.

In a review of 2499 histologically confirmed canine neoplasms, Fölger<sup>9</sup> found but 3 "melanosarcomas." Among 31 multiple primary tumors in dogs, Cohrs<sup>10</sup> observed, adjacent to the left eye of a 14-year-old male dachshund, a cutaneous melanoma with transformation to spindle cell sarcoma. A "melanosarcoma" of a mammary gland, with metastases in the brain and spinal cord, was described by Feldman<sup>11</sup> in a group of 17 canine tumors. Among 15 dogs with microscopically verified neoplasms, Chambers<sup>12</sup> reported the case of a 10-year-old male Aberdeen terrier with "melanotic sarcoma" of the inguinal region. Of the 336 histologically examined canine tumors in the large series of Auler and Wernicke,<sup>13</sup> only one was a "melanosarcoma." Passey<sup>14</sup> was

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able to produce malignant melanoma in the dorsal cervical region of 2 mongrel Airedale bitches by applying tar weekly for about 7 years.

McClelland<sup>15</sup> mentioned a melanoma in a young male Doberman-Pinscher examined by Fehr and Wilder.<sup>16</sup> He also described 2 cases of his own. The first was an 8-year-old male chow which had a malignant melanoma of the metacarpal region with metastases to popliteal, inguinal, and mesenteric lymph nodes and to the lungs. The second concerned a 10-year-old male chow with a malignant melanoma of the tongue which metastasized to cervical lymph nodes, mediastinum, pleura, and lungs. He also had seen 2 aged Boston terriers each afflicted by melanoma of the choroid of one eye.

Bloom<sup>17</sup> recorded the case of a 1½-year-old male chow which had a "melanocarcinoma" of the iris of the right eye.

#### DEFINITION OF TERMS

The term "noncancerous melanoma" will be employed to designate the benign neoplasm of cells capable of elaborating pre-melanin (melanoblasts) or melanin (melanocytes). The term "nevus" is objectionable, since it means simply a "blemish," usually of congenital origin, and may be derived from endothelial cells, pigment-forming cells, or squamous epithelial cells. "Melanocytoma" is inappropriate, for this name may include all cells containing melanin, such as macrophages and the cells of the bottom row of the epidermis. Although melanoma has been used to mean a "malignant" neoplasm of cells capable of forming melanin within themselves, this is to be deplored as much as if fibroma or chondroma were similarly abused.

The term "cancerous melanoma" will be used to designate the anaplastic, invasive, and metastasizing neoplasm derived from cells capable of forming melanin. "Cancer" has been established by long usage as a name for neoplasms of any type which are "malignant" or "non-benign" or anaplastic, invading, and metastasizing, although some still restrict its use to designate an anaplastic epithelial growth, namely, carcinoma. Consequently, "malignant" has been avoided, even though it means "acting maliciously." The designations "melanocarcinoma" and "melanosarcoma" have been shunned. Usually these terms simply indicate that polyhedral cells or spindle cells preponderate in a given cancerous melanoma, whereas mixtures of the two in varying proportions are often found when enough sections of the primary neoplasm and/or its metastases have been examined. Some workers believe that melanoblasts, the basic cells of "melanoma," are of ectodermal origin, whether through migration of neural crest cells into the skin or from

primary origin in the epithelial cells of the epidermis. Thus, "melanocarcinoma" might be suitable, but gliomas and nerve sheath tumors should then be grouped under "neurocarcinoma," an obviously objectionable procedure. If the term "melanosarcoma" were used, then the evidence supporting an ectodermal origin for melanoblasts must be disregarded. However, this term might be acceptable when the close relation between the embryonic development of nerve sheath cells and melanoblasts is taken into account, for the cancerous variant of the nerve sheath tumor (neurofibroma) may be properly labeled "neurofibrosarcoma."

#### MATERIALS AND METHODS

The neoplasms examined were obtained from several veterinarians.\* The tissues were surgically resected in 22 cases, were obtained at reasonably complete autopsy in 7 cases, and were excised after death in 2 cases. They were fixed in 4 per cent formaldehyde and routine tissue sections, stained by hematoxylin and eosin, were prepared as previously described.<sup>18</sup> Becker's silver nitrate stain<sup>19</sup> for melanin and Turnbull's blue reaction for iron-containing pigment were used on several neoplasms. A few heavily pigmented lesions were bleached by potassium permanganate and oxalic acid and were then stained routinely. The dopa reaction was not done, because dihydroxyphenylalanine was not available during the course of this study.

#### SUMMARY OF CASES

The cases will be listed in the order in which they were received in this laboratory for study and diagnosis.

*Case 1 (MP-5760).* A male Boston terrier, 12 years old, had a 30 by 13 by 8 mm., dark brown, gray mottled, firm, noncancerous melanoma, covered by ulcerated skin, in the right axilla.

*Case 2 (46R-47).* A female Chihuahua, 9 years old, had multiple cutaneous tumors at autopsy. Two submitted for study were noncancerous melanomas measuring 15 by 8 by 3.5 mm. and 25 by 15 by 1.5 mm.

*Case 3 (46R-146).* A male Airedale terrier, 14 years old, had many dark brown tumors on the tail. The one studied measured 17 by 13 by 12 mm. and was a firm, dark brown, skin-covered, noncancerous melanoma with a 2 mm. pedicle. Also present was a 26 by 17 by 13 mm., pink-gray, red mottled, ulcerated, cancerous melanoma of the right buccal region.

*Case 4 (46R-156).* A female Pekingese, 12 years old, presented a 10 mm., firm, gray, brown-streaked, noncancerous melanoma of a left eyelid.

\* Drs. L. R. Phillips and B. S. Burkhardt of Lakewood, Colorado (10 cases), Dr. Rue Jensen of Colorado A. and M. College (9 cases), Drs. D. B. LyVere and L. S. Peavy of Denver (2 cases each), and Drs. M. H. Camner, F. T. Candlin, C. L. Davis, and J. R. Naylor of Denver, Dr. J. R. Durigg of Aurora, Colorado, Drs. G. H. Gilbert and V. D. Stauffer of Arvada, Colorado, Drs. J. C. Flint and R. A. Nipko of Salt Lake City, and Dr. W. J. Zontine of Lancaster, California (1 case each).

*Case 5 (47R-5).* A male German shepherd, 14 years old, had an 8 mm., firm, dark brown, noncancerous melanoma in the left metacarpal region.

*Case 6 (47R-7).* A female Boston terrier, 9 years old, presented a firm, black, skin-covered, noncancerous melanoma on the right hock, measuring 11 by 9 by 7 mm.

*Case 7 (47R-174).* On a male Scotch terrier, 14 years old, there were a firm, black, noncancerous melanoma of the left lower eyelid, which measured 18 by 8 by 4 mm., and an irregular, firm, black, cancerous melanoma in the right temporal region, measuring 20 by 17 by 9 mm.

*Case 8 (47R-311).* A male English setter, 14 years old, had a cancerous melanoma on the posterior wall of the pharynx, from which 32 gm. of tissue were submitted for examination. The dog was unable to swallow even liquids and was emaciated and dehydrated.

*Case 9 (47R-314).* A male Belgian shepherd, 11½ years old, presented two cancerous melanomas along the left costal margin. The first was 50 by 33 by 21 mm., firm, black and gray mottled, and had been present for 3 months. The second was 95 by 65 by 35 mm., pale yellow and gray, firm, divided into nodules 1 to 15 mm. in diameter, invaded the overlying skin, and had been present for 9 months.

*Case 10 (47R-353).* A female Airedale, 10 years old, had two noncancerous melanomas: one, 20 by 20 by 15 mm., pedunculated, covered by ulcerated skin, firm, gray and black mottled, was in the sternal region; the other, 50 by 35 by 30 mm., fairly soft to firm, and jet black, was in the left posterior phalangeal region.

*Case 11 (47R-384).* A male cocker spaniel, 7 years old, had had a firm, black, noncancerous melanoma, marked by a 2 mm. gray area, at the outer canthus of the left eye for 2 months. It measured 12 by 7 by 6 mm.

*Case 12 (47R-487).* A female mongrel Boston terrier, 10 years old, had had a 47 by 36 by 35 mm., firm, coarsely lobulated, gray and black mottled, cancerous melanoma covered by ulcerated skin for 3 months. Clinical evidence of metastases to the regional lymph nodes was observed 5 months after wide excision.

*Case 13 (47R-518).* A male Scotch terrier, 5 years old, presented a firm, gray, brown mottled, cancerous melanoma, measuring 15 by 10 by 10 mm., which had invaded the bone and marrow of a phalanx of the left forefoot.

*Case 14 (47R-614).* A male English springer spaniel, 13 years old, had an 18 mm., firm, gray, cancerous melanoma of the buccal region with metastases in the cerebral hemispheres. One metastasis was 9 mm. and dark brown; the other, 11 mm. and gray.

*Case 15 (47R-646).* A male English springer spaniel, 11 years old, had a cancerous melanoma, site unspecified, and metastases up to 2 cm. in diameter in the omentum, spleen, and lungs. Only the metastases were available for histologic examination.

*Case 16 (47R-654).* A female collie, 10 years old, had a cancerous melanoma of the nictitating membrane of one eye, measuring 10 by 10 by 5 mm.

*Case 17 (47R-659).* A male Irish water spaniel, 11 years old, had a 25 by 20 by 10 mm. cancerous melanoma, site unspecified.

*Case 18 (47R-685).* A male shepherd dog, 6 years old, presented a soft to firm, tan, dark red mottled, cancerous melanoma, measuring 32 by 25 by 24 mm., which had developed over the right costal margin during 6 months.

*Case 19 (47R-690).* A male cocker spaniel, 7 years old, had had a 12 by 9 by 5 mm. cancerous melanoma in the left gluteal region for 4 months.

*Case 20 (47R-718).* A spayed female Airedale, 12 years old, had a firm, dark brown, cancerous melanoma, weighing 935 gm., of the left hind leg. There were dark brown metastases in lymph nodes (left inguinal, 130 gm.; pelvic, 300 gm.; and thoracic, 330 gm.), heart, lungs, pancreas and kidneys, and, to a lesser degree, in the liver, ileum, and pleura. The brain contained no metastases. Four years before euthanasia, the primary neoplasm had been noted on the left hock and it gradually

spread to involve the posterolateral aspect of the tibial region and the distal third of the thigh. Clinically, emaciation and great enlargement of the inguinal lymph nodes were noted.

*Case 21 (48R-28).* A male English springer spaniel, 11 years old, had a cancerous melanoma, site unspecified, with multiple metastases in lungs and spleen. Only the metastases were available for histologic examination.

*Case 22 (48R-31).* A female Scotch terrier, 10 years old, presented a cancerous melanoma in the left thoracic region with metastases up to 2 cm. in diameter in the lungs, spleen, and kidneys. Only the metastases were available for histologic study.

*Case 23 (48R-59).* A female Scotch terrier, 9 years old, had had a spongy, pale yellow and dark brown mottled, pedunculated, ulcerated, noncancerous melanoma of the left thoracic region for 1 year. It measured 37 by 32 by 19 mm.

*Case 24 (48R-130).* A male Scotch terrier, 6 years old, had had a 10 by 4 by 2 mm., firm, gray-black, noncancerous melanoma of the left inner canthus for 6 months.

*Case 25 (48R-162).* A male cocker spaniel, 13 years old, had a firm, dark gray-brown, cancerous melanoma of the buccal region, which measured 25 by 22 by 15 mm.

*Case 26 (48R-180).* A male German shepherd, 10 years old, had a 6 by 5 by 4 mm. noncancerous melanoma of the right inner canthus.

*Case 27 (48R-203).* A female English springer spaniel, 4 years old, had a firm, black, noncancerous melanoma, measuring 8 by 7 by 5 mm., of the left lower eyelid next to the medial canthus.

*Case 28 (48R-231).* An old dog of unknown sex and breed had a 2 by 1 by 1 cm., firm, black, noncancerous melanoma of an eyelid.

*Case 29 (48R-235).* A female Scotch terrier, 3 years old, had a cancerous melanoma over the costal margin with metastases in the lungs and brain up to 1 cm. in diameter.

*Case 30 (48R-251).* A male Scotch terrier, 13 years old, had had for several months a firm, gray and brown mottled, cancerous melanoma, weighing 13 gm., of the left buccal region.

*Case 31 (48R-267).* A male Boston terrier, 14 years old, had a noncancerous melanoma on the tip of the right ear for more than a year. It measured 10 by 7 by 5 mm.

#### ANALYSIS OF CASES

Statistics on sex, age, and breed are based on the 30 dogs about which these data were known. Nineteen were males. In only 3 was the age less than 6 years, the remainder being 6 to 14 years old. The breeds represented included: terrier, 14 (Scotch, 7; Boston, 4; Airedale, 3), spaniel, 8 (English springer, 4; cocker, 3; Irish water, 1), shepherd, 4, and Chihuahua, Pekingese, English setter, and collie, 1 each. All of these breeds, with the exception of the Chihuahua, have heavily or moderately pigmented skin. The absence of the fox terrier from the dogs affected by melanomas is probably significant, since this breed, both smooth-coated and wire-haired, has relatively nonpigmented skin, but is often subject to other spontaneous neoplasms.<sup>20</sup> These data indicate that the breeds with more heavily pigmented skin are more susceptible to melanoma, as contrasted to humans, among whom those with lightly pigmented skin tend to be more often affected by melanoma. Of the 36 melanomas available for study, as primary growths or as metas-

tases, or as both, 17 were noncancerous and 19 were cancerous. Of these 36, 14 were located on the head, 8 on the thorax, 7 on the extremities, and 1 each on the neck and tail. The location of 5 tumors was not specified. Particularly notable was the predilection of the primary growth for the eyelids in 8 cases and for the cheeks in 4 cases. These 36 melanomas accounted for more than 5 per cent of the canine neoplasms studied in this laboratory.

### *Gross Findings*

The dimensions or weight of 32 primary melanomas were available. By rough calculation, 15 of the 17 noncancerous tumors had a volume of 80 to 6000 cmm., and the 2 remaining, 22,496 and 52,500 cmm. Nine of 15 cancerous melanomas with available data had a volume of 8250 to 935,000 cmm. and the 6 remaining, 540 to 5832 cmm. These figures indicated that a given melanoma had a 70 per cent chance of being noncancerous if its volume were 6 cc. or smaller and an 80 per cent chance of being cancerous if its volume were 8 cc. or greater.

The primary neoplasms were covered by skin, which usually was heavily or moderately pigmented and often was ulcerated in small (Fig. 1) or large (Fig. 2) areas. Sometimes the tumor was pedunculated (Fig. 3). The cut section was spongy, fairly firm, and varied from relatively uniform dark brown (Fig. 1) to brown mottled with gray or yellow (Fig. 3), and sometimes even to jet black. Lobulation was fairly common in the larger tumors (Figs. 2 and 4), which were composed of dark brown, gray-brown, and gray nodules of varying size. An exceptionally large melanoma (Fig. 5), which produced many metastases (Fig. 6), was diffusely dark brown and, like other heavily pigmented growths (Fig. 1), left a slimy brown fluid on the knife on sectioning. Two or more primary melanomas were found in 4 dogs. At autopsy, metastases (Fig. 6) were observed in 6 dogs, 5 times in the lungs, 3 in the spleen, 2 in the kidneys, 2 in the brain, and once each in the lymph nodes, heart, ileum, liver, pancreas, omentum, and pleura. The metastases varied from 1 to 5 mm. up to 20 to 25 mm. in diameters.

### *Microscopic Examination*

The noncancerous melanomas consisted of polyhedral cells (Fig. 7), of stubby or long spindle cells (Fig. 8), or of tapered slender cells. Also quite frequent were cells with multiple, thin, dendritic processes. The polyhedral cells tended to be grouped in solid masses, in small alveolar patterns, or in larger compartments. The long spindle cells and the tapered slender cells were usually arranged in sweeping bundles, were

sometimes mingled with small nerve trunks in the subcutaneous tissue, and resembled proliferated nerve sheath cells, especially in areas where some nuclear palisading was noted. As a rule, the cells of noncancerous melanomas were set together in rather close-knit fashion (Figs. 9 and 10), except in areas of edema or near areas of ulceration, in which they were spread apart. Whorls of cells were fairly frequent (Fig. 10) and recalled the structure of Meissner's corpuscles. The amount of acidophilic cytoplasm varied, but was notably conspicuous in the polyhedral forms. The ratio between cytoplasmic and nuclear volume was fairly constant, considering the shapes of the cells. Melanin pigment was scanty or absent and the nuclei were easily seen (Figs. 9 and 10) or was so abundant that the nuclei of the cells were practically obscured (Figs. 7 and 8), so that bleaching was necessary in order to see the nuclei. The cells with several thin, dendritic processes were usually heavily laden with melanin granules. The nuclei were almost invariably single, were round or oval in the polyhedral cells, and were oval to oblong in the long spindle and slender tapered cells. Nuclei of a given shape were quite close to each other in size. The chromatin net (Figs. 9 and 10) was fine and homogeneous, was distributed evenly in the nucleus, and filled the entire nucleus, which usually had a sharply demarcated border and contained a definite, nearly always single, small nucleolus. Occasional or scattered, regular mitotic figures were noted, especially when the cells were near areas of inflammation at the surface of an ulcerated neoplasm. The overlying epidermis, when intact and when distant from the denuded areas, revealed granules of melanin pigment, moderate to heavy in the bottom row of epidermal cells and in nearly all of the cells of the epidermis in some. This epidermal pigmentation was noted also in the cancerous melanomas.

The shape of the cells in the cancerous melanomas was similar to that of the cells in the noncancerous tumors, but polyhedral and stubby spindle cells predominated (Figs. 11 and 12). Forms with dendritic processes were rare or absent. In areas free of infection or edema the cells tended to have a much looser arrangement. They usually were massed, alveolar, or in compartments, or, less commonly, in short bundles. The tendency to resemble nerve sheath tumors in some areas was not a feature of the cancerous melanomas. The cells, as a rule, were larger and had relatively less acidophilic cytoplasm, which in some areas of a few neoplasms tended to be clear and to contain pyknotic nuclei. The nuclei were much larger in relation to the volume of the cytoplasm than in the noncancerous melanomas. The amount of melanin in the cells was of little aid in the differentiation of noncancerous from can-

cerous melanomas, although the cells of the cancers with the more anaplastic characteristics (Fig. 12) tended to inclose little or no melanin. In most of the others (Fig. 11), the melanin varied from slight to abundant, but was hardly ever so plentiful as to obscure the nuclei. The nuclei usually were round or oval and plump, and varied widely in size, some being giant and bizarre (Figs. 13 and 14). The nuclear chromatin was coarse, unevenly distributed, and tended to occupy a peripheral position in the nucleus and to cause an apparent thickening of the perinuclear membrane by its margination. Nucleoli were enlarged and prominent; sometimes 2 or 3 were present. Deep invasion of the subcutaneous adipose tissue, of skeletal muscle, or lymphatics, and of veins was noted with several cancerous melanomas. Areas of necrosis and hemorrhage were fairly common. The microscopic features of the metastases were similar to those of the primary neoplasms, when both were available for study.

#### SUMMARY

Of 36 melanomas observed in 31 dogs, 17 were noncancerous and 19 were cancerous.

In the 30 dogs in which sex and age were known, 19 were males. Only 3 were less than 6 years old, the remainder being 6 to 14 years.

The breeds chiefly affected were those with heavily or moderately pigmented skin.

Melanomas of the head, thorax, and extremities accounted for 29 of the neoplasms observed. The predilection of the primary growth for eyelids (8 cases) and for cheeks (4 cases) was especially noteworthy. Four dogs had two or more primary melanomas. Six dogs showed metastases at autopsy, the lungs being affected in 5.

The melanomas with a volume of 8 cc. or more had about an 80 per cent chance of being *cancerous*. The melanomas with a volume of 6 cc. or less had about a 70 per cent chance of being *noncancerous*.

The looseness of cell arrangement, the preponderance of polyhedral and stubby spindle cells, the large size of nuclei in relation to cell volume, the increased number of round and oval nuclei, the variation in size of nuclei, multiplicity of nuclei, coarsening and margination of chromatin, and increase in size and number of nucleoli were all of considerable aid in differentiating cancerous from noncancerous melanoma. In this differentiation, mitotic figures were of some help, if they were numerous and irregular or pluripolar. The amount of melanin in the neoplastic cells varied in different areas of both noncancerous and cancerous melanoma. A tendency for lessened melanin production was observed in the more anaplastic cells of a given cancerous melanoma. Local invasion

of adipose tissue, skeletal muscle, lymphatics, and veins, and metastasis afforded ultimate evidence that a melanoma was cancerous.

It is considered that good reasons exist for designating the innocent neoplasm of melanin-forming cells as "noncancerous melanoma" and for the application of the name "cancerous melanoma" to the anaplastic, invasive, metastasizing neoplasm of melanin-forming cells.

Mr. Glenn E. Mills gave technical assistance with photography.

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## DESCRIPTION OF PLATES

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### PLATE 44

- FIG. 1. Case 10. Noncancerous melanoma. The skin surface is shown in the upper figure. Lighter areas are locally ulcerated. The homogeneous, dark brown cut surface is illustrated below.
- FIG. 2. Case 12. Cancerous melanoma. The skin surface in the upper figure is ulcerated over its superior portion; and the line of excision of the broad base appears in the inferior portion. The nodular, brown, gray mottled cut surface is shown in the lower figure.
- FIG. 3. Case 23. Noncancerous melanoma. Skin surfaces are shown in the upper and lower figures. The pedicle appears in the upper illustration. The brown and pale yellow mottled cut surface is shown in the middle figure.
- FIG. 4. Case 9. Two cancerous melanomas with nodular, brown and gray mottled cut sections. Skeletal muscle is invaded at the lower edge of the larger neoplasm.

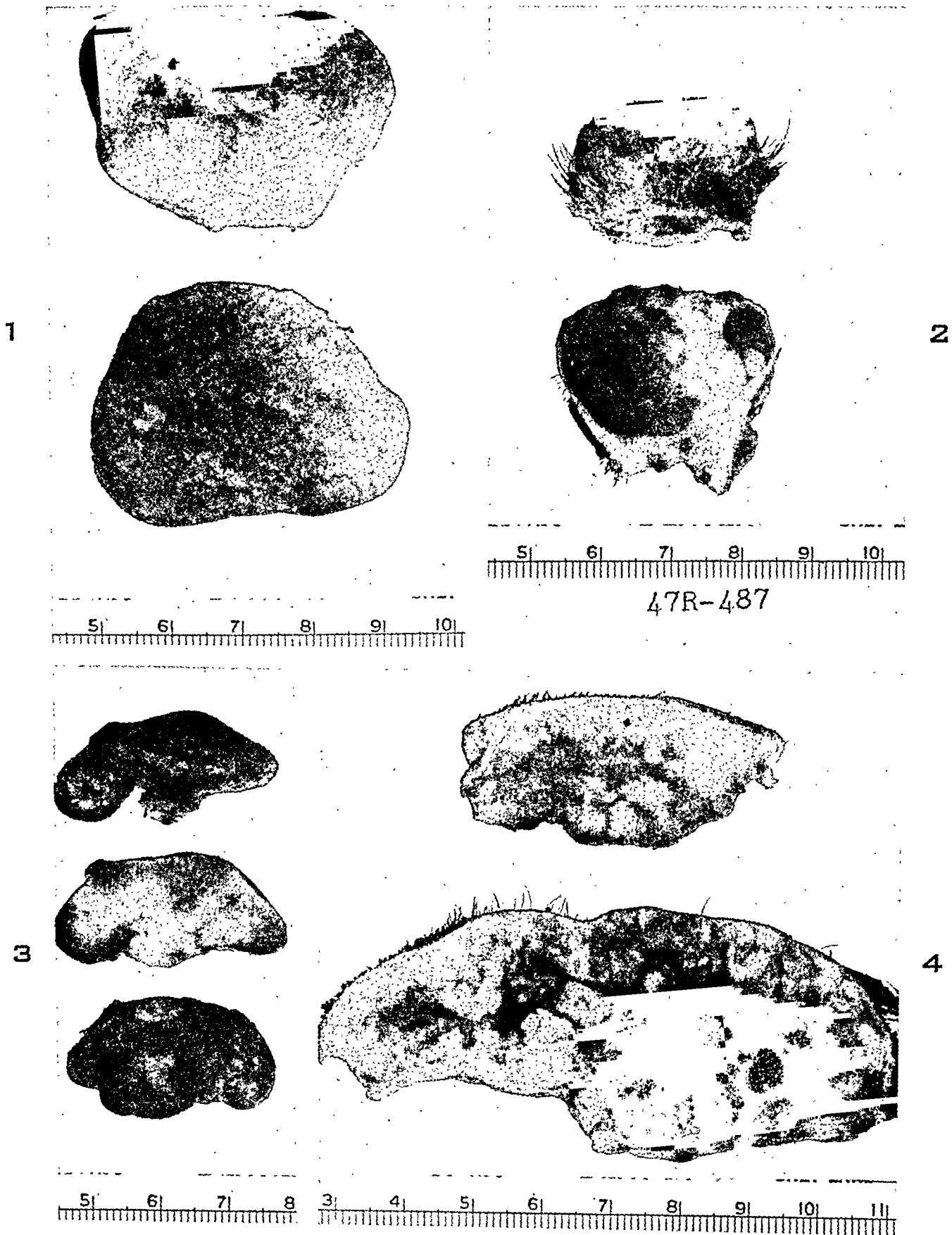


PLATE 45

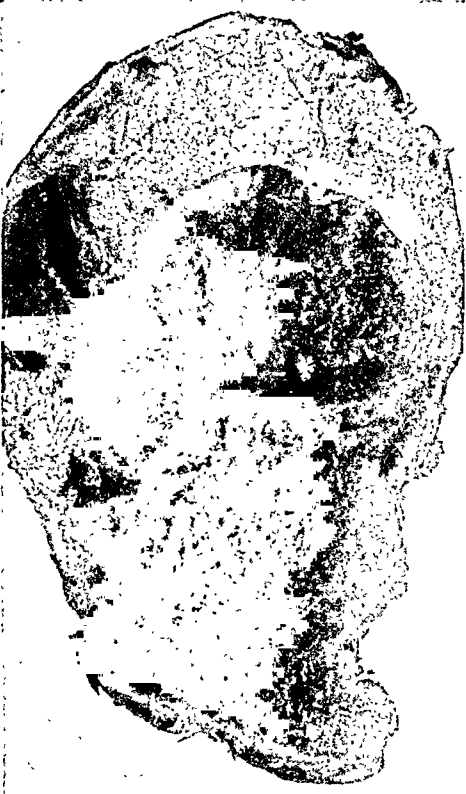
FIG. 5. Case 20. Cancerous melanoma, lateral aspect, with a homogeneous, deep brown surface after sectioning. The lower skin-covered border was in the region of the hock and the upper fascia-covered border in the region of the thigh.

FIG. 6. Case 20. Metastases of cancerous melanoma in lung and kidney. Metastases in thoracic and inguinal lymph nodes and in pancreas are shown also (viewed from left to right in the lower part of the picture).

FIG. 7. Case 5. Noncancerous melanoma. Melanin is present in the cells of all layers of the epidermis at the left. Masses of melanin-laden, polyhedral tumor cells in the dermis and upper hypodermis. Hematoxylin and eosin stain.  $\times 150$ .

FIG. 8. Case 6. Noncancerous melanoma. In a rete peg of hypertrophied epidermis in the lower left-hand corner, the epithelial cells contain much melanin. Melanin-laden, long spindle-shaped tumor cells are abundant in the dermis and upper hypodermis. Hematoxylin and eosin stain.  $\times 150$ .

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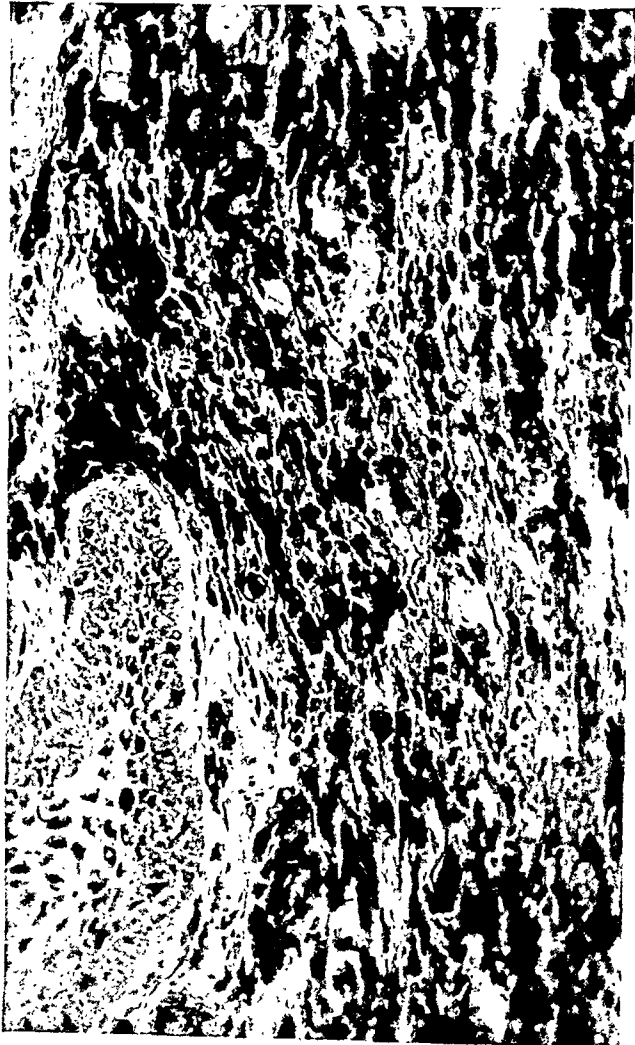


PLATE 46

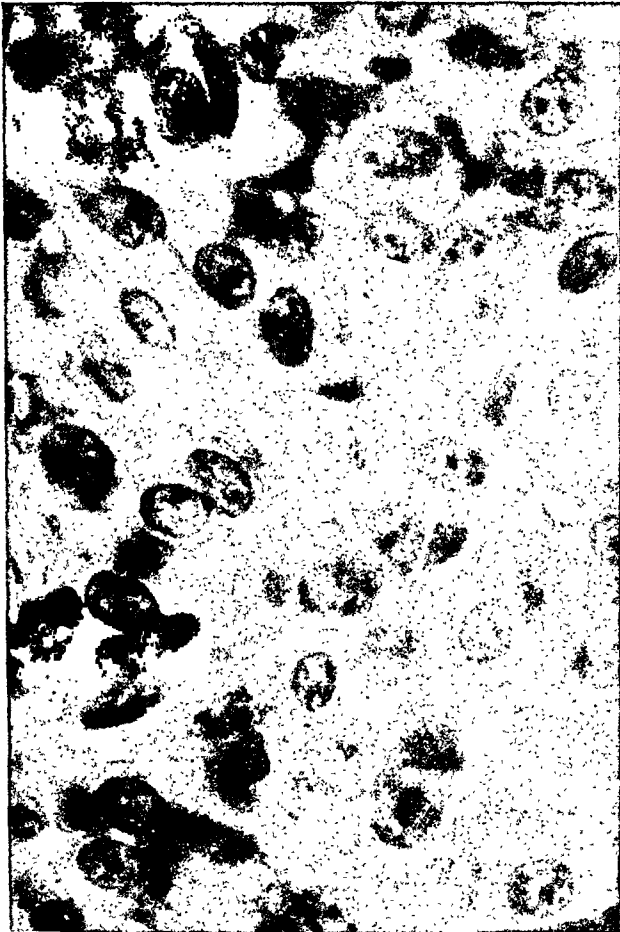
FIG. 9. Case 11. Noncancerous melanoma with compactly arranged polyhedral and stubby spindle-shaped cells containing no melanin. The nuclei are round or oval. The chromatin net is regular, fine, and homogeneous. Nucleoli are small and discrete. Hematoxylin and eosin stain.  $\times 900$ .

FIG. 10. Case 11. Noncancerous melanoma. Stubby and long spindle-shaped cells predominate in this field. Melanin is moderate to abundant. The whorled arrangement just below and to the left of the center suggests a Meissner's corpuscle. There are fibrillar cellular processes in the lower portion of the field. Hematoxylin and eosin stain.  $\times 900$ .

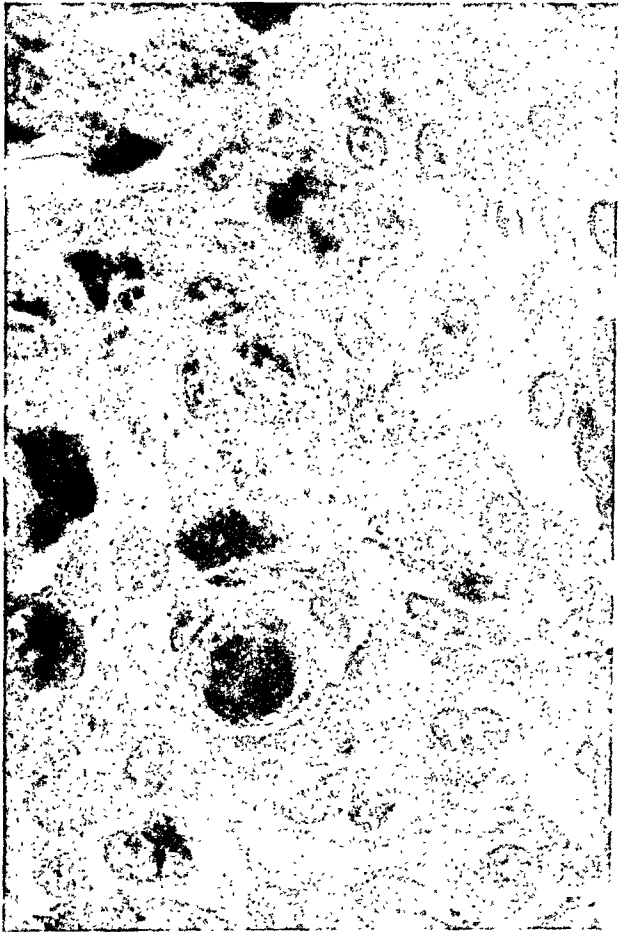
FIG. 11. Case 7. Cancerous melanoma showing a loose arrangement of stubby spindle-shaped and polyhedral cells. Melanin content and nuclear sizes are variable. The chromatin is coarse and irregular and the nucleoli are enlarged and prominent. Hematoxylin and eosin stain.  $\times 450$ .

FIG. 12. Case 3. Cancerous melanoma with stubby spindle-shaped cells loosely arranged and containing no melanin. The nuclei are plump and the chromatin, coarse and uneven. Nucleoli are enlarged. Hematoxylin and eosin stain.  $\times 450$ .

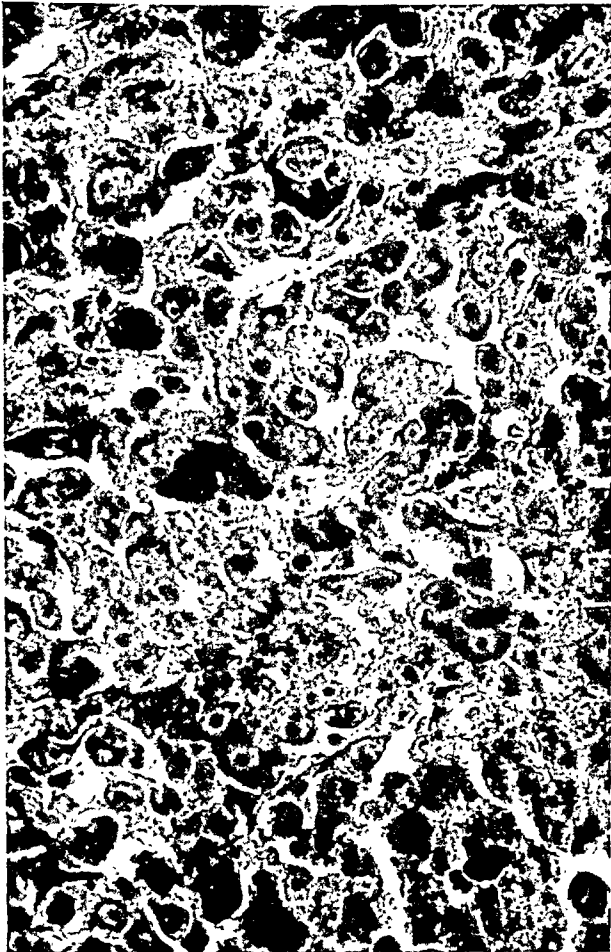
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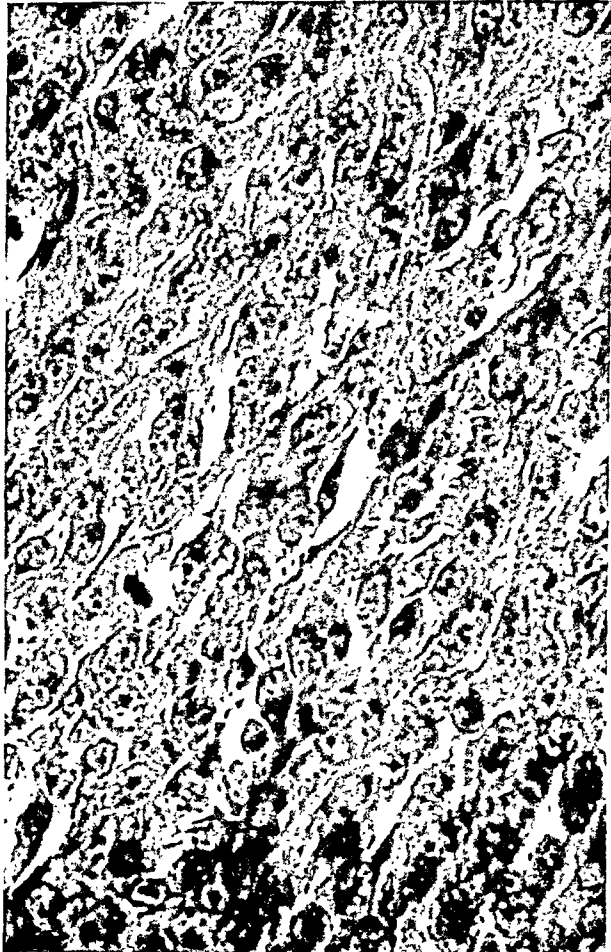


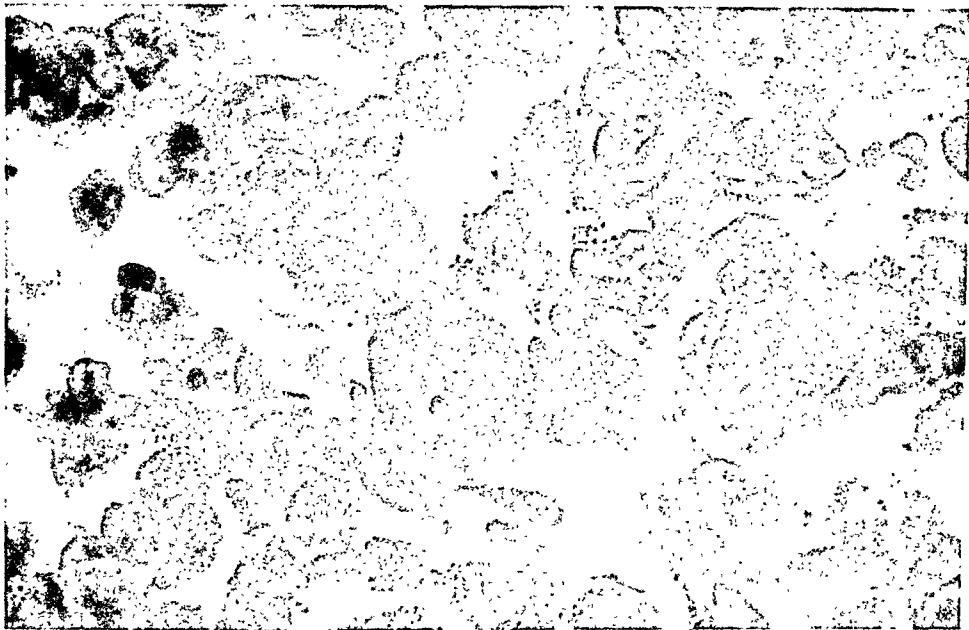
PLATE 47

FIG. 13. Case 7. Cancerous melanoma showing a loose arrangement of stubby spindle-shaped and polyhedral cells. Melanin content and nuclear sizes are variable. The chromatin is coarse and irregular and the nucleoli are enlarged and prominent. Hematoxylin and eosin stain.  $\times 900$ .

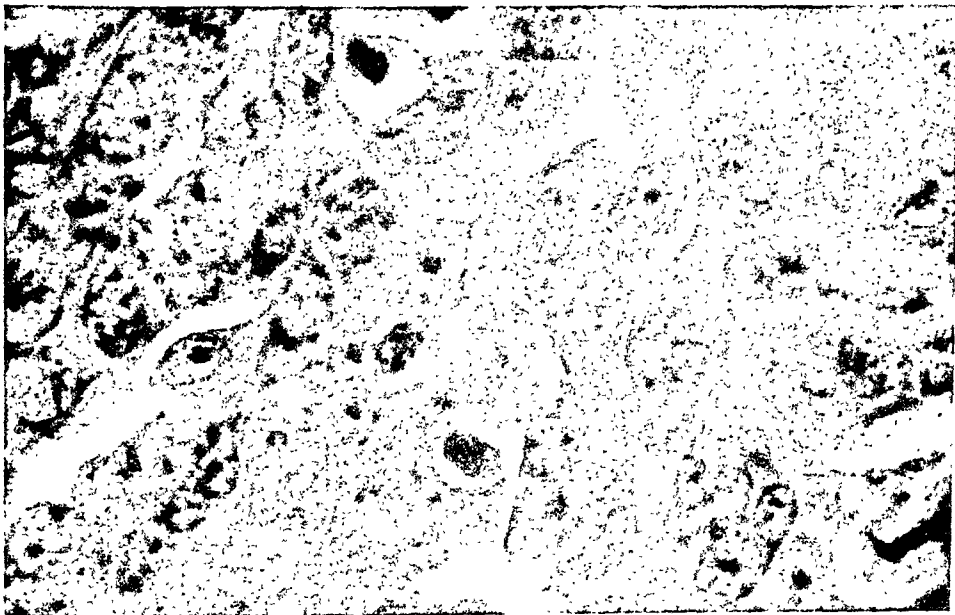
FIG. 14. Case 3. Cancerous melanoma with stubby spindle-shaped cells loosely arranged and containing no melanin. The nuclei are plump and the chromatin, coarse and uneven. Nucleoli are enlarged. Hematoxylin and eosin stain.  $\times 900$ .

FIG. 15. Case 3. Cancerous melanoma with stubby spindle-shaped cells loosely arranged and containing no melanin. The nuclei are plump and the chromatin, coarse and uneven. Nucleoli are enlarged. Of note is the plump mitotic figure just below the right of the center. Hematoxylin and eosin stain.  $\times 900$ .

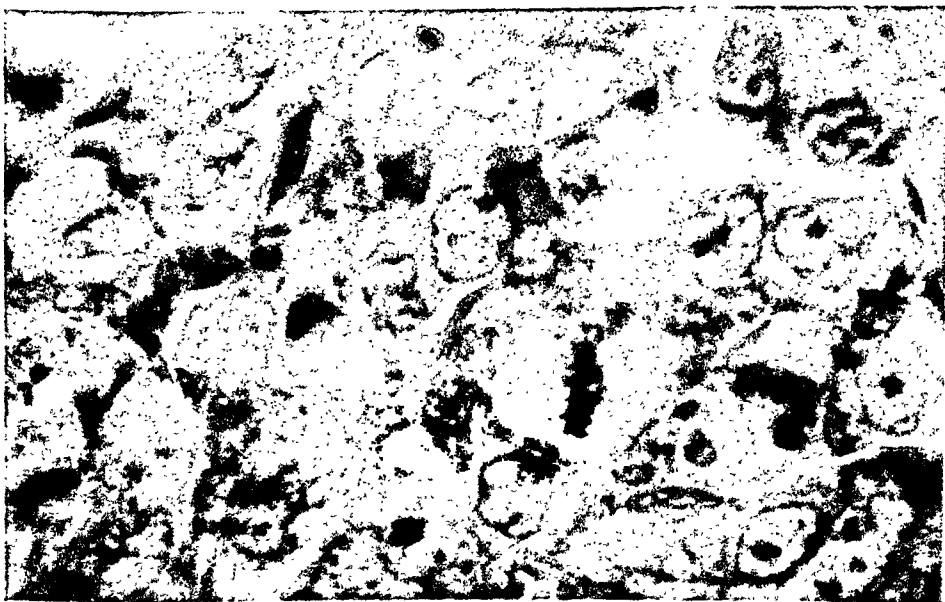
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## CARDITIS IN POLIOMYELITIS AN ANATOMIC STUDY OF THIRTY-FIVE CASES AND REVIEW OF THE LITERATURE \*

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This investigation was undertaken because of the relative paucity of reports in the literature regarding cardiac lesions in cases of acute poliomyelitis and because we observed certain unusual cardiovascular lesions during the 1946 epidemic in Minnesota.

In 1910 Robertson and Chesley reported on the necropsies in 6 cases of acute poliomyelitis. In 5 the hearts were studied histologically and swelling of myocardial fibers and interstitial edema of the myocardium were noted. Even then these authors called attention to the lack of emphasis in the literature upon the changes in organs outside the nervous system.

Minimal myocardial inflammatory changes in cases of acute poliomyelitis were subsequently reported by Abramson, in 1918, and by Landon and Smith, in 1934. The latter authors stated that the observed lesions were similar to those occurring in diphtheria and scarlet fever, but apparently they did not feel that the changes were significant.

In 1934 Cowie, Parsons, and Lowenberg, reporting necropsy findings in 4 cases of acute poliomyelitis, included one case in which localized myocarditis of the right atrium, a mural thrombus at this site, and pulmonary emboli were observed.

Clark, in 1938, reported data on an interesting case of acute poliomyelitis in which severe diffuse interstitial myocarditis was found at necropsy. Since horse serum had been administered, with the subsequent development of typical signs of serum sensitivity, the myocarditis was attributed to horse serum.

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It remained for Saphir and Wile, in 1942, to point out that myocarditis is a feature of poliomyelitis. The histologic changes described by these authors in 6 of 7 cases included cellular exudate in the myocardium, consisting mainly of monocytes, adventitial cells, and lymphocytes, or of neutrophils and lymphocytes. Minimal degenerative changes also were described. Peale and Lucchesi, in 1943, reported the finding of essentially similar changes in 5 of 9 cases. In the same year Dublin and Larson reported the finding of 2 incidental cases of acute myocarditis among 12 cases of fatal acute poliomyelitis.

In 1945 Saphir published the results of a necropsy study on 17 additional cases of acute poliomyelitis, in 10 of which he found myocarditis. In 3 cases he noted histologic changes in the media of the aorta, consisting of separation of elastic lamellae by a substance which, he stated, had a fibrinoid appearance.

Luhan, in 1946, reported a series of 13 cases of fatal acute poliomyelitis, in one of which he found verrucous endocarditis involving the mitral valve. The heart was not further described.

Recently Gefter, Leaman, Lucchesi, Maher, and Dworin published a clinical and pathologic study of a group of 467 cases of acute poliomyelitis, in 6 of which necropsy was performed. Their investigation disclosed interesting facts concerning the incidence of cardiac murmurs, electrocardiographic changes, and other pertinent clinical findings related to the heart. Thirty-two (14.2 per cent) of 226 patients were found to have abnormal electrocardiograms. Myocarditis was found in 2 of the 6 cases in which necropsy was performed. Unfortunately, it had not been possible to take electrocardiographic tracings on these patients, because of the severity of their illnesses.

A survey of the literature on the pathology of poliomyelitis does not permit accurate determination of the incidence of significant cardiac changes, since the histopathologic findings in the heart are seldom described in the reports. After review of the few available studies in which the heart was specifically investigated, it would seem that cardiac lesions are common in cases of fatal acute poliomyelitis.

#### MATERIAL AND METHODS

The 35 cases used for the investigation herein reported represent all of the cases of poliomyelitis in which necropsy was performed at the Mayo Clinic, from August 2, 1925, to December 11, 1946, inclusive, and in which the hearts were available for this study. The specimens were sectioned for histologic investigation by the method used by Gross, Antopol, and Sacks in the study of rheumatic heart disease. Their method

was adopted primarily because it made possible, by simple standardized technics, the demonstration of most of the important anatomic structures of the heart. The hearts had all been fixed in 10 per cent formalin. Six blocks were cut from each specimen and embedded in paraffin. Sections were cut at 4 to 6  $\mu$ . Hematoxylin and eosin stains were used on sections from each block. Bodian's stain and Mallory's phosphotungstic acid hematoxylin stain were used in selected cases to demonstrate changes in muscle fibers. Where specifically indicated in order to evaluate changes in connective tissue, Verhoeff's elastic tissue stain counterstained with van Gieson's stain was employed. McCallum's Gram stain was used to demonstrate the presence or absence of bacteria. Blocks from selected specimens were stained with sudan III to determine whether fatty degeneration was present.

## ANATOMIC OBSERVATIONS

### GROSS FINDINGS

The hearts usually were moderately increased in weight, as shown in Table I. No significant changes in color or consistency at the time of necropsy were recorded.

Slight to moderate dilatation of the ventricles was found in 24 hearts. Usually only the right ventricle was dilated, but left ventricular dilatation accompanied the more severe degrees of right ventricular dilatation. Significant ventricular hypertrophy was found in only 3 hearts.

There were unusual gross findings in 3 cases in which myocarditis was demonstrated microscopically. One heart (case 1) presented a perforation of the posterior wall of the right atrium. The defect measured about 3 by 4 mm., was oval, and had slightly irregular borders (Fig. 1). There was hemorrhage into the surrounding tissue and there were about 100 cc. of bloody fluid in the pericardial sac.

Verrucous endocarditis of the mitral valve was found in another heart (case 12). There were firm, grayish red, slightly irregular vegetations situated along the line of closure of the mitral leaflets (Fig. 2). No old rheumatic stigmas were found.

There was verrucous endarteritis of a patent ductus arteriosus in a third case (no. 13). The vegetations were friable, brownish red, elevated lesions protruding from the pulmonary end of the ductus arteriosus (Fig. 3).

### HISTOLOGIC FINDINGS

#### *Myocarditis*

Histologic evidence of myocarditis was found in 14 hearts. The myocardial changes varied considerably in extent and severity. Lesions were

somewhat more frequent in the left posterior papillary muscle and the ventricular septum than in other parts of the heart.

In 3 hearts (cases 1, 2, and 3) minimal acute myocarditis was present. Degenerative changes included irregular affinity of the cytoplasm of

TABLE I  
*Heart Weights: Comparison with Normal Standards\**

Case	Age	Sex	Estimated body weight	Heart weight	Normal heart weight according to sex and age, or sex and body weight
	<i>years</i>		<i>lbs.</i>	<i>gm.</i>	<i>gm.</i>
1	7	M		120	93.3
2	9	M		165	108.3
3	15	M		330	200.6
4	21	M		335	303.5
5	22	M		228	311.1
6	26	M	150	300	294.0
7	36	F	165	222	297.0
8	21	F		235	250.6
9	31	M	140	295	274.0
10	32	F	150	340	272.0
11	44	M	185	383	363.0
12	34	M	165	340	297.0
13	7	F		140	81.4
14	17	M		336	250.9
15	17	M		250	250.9
16	31	F	120	163	215.0
17	3	M		70	64.5
18	3	M		85	64.5
19	4	M		75	74.7
20	5	M		109	83.7
21	5	M		96	83.7
22	10	M		173	130.9
23	10	M		130	130.9
24	12	M		143	157.0†
25	13	F		253	142.5
26	14	M		267	216.1
27	14	F		161	173.8
28	17	M		353	250.9
29	17	M		315	250.9
30	20	M		355	305.3
31	22	M		382	311.1
32	22	M		310	311.1
33	23	F		220	258.5
34	29	M	155	290	304.0
35	30	M	170	370	333.0

\* Normal heart weights from Vierordt and Smith.

† Mean normal heart weight.

muscle fibers for eosin, swelling of muscle fibers, loss of striations, cytoplasmic vacuolation, and occasional fragmentation of muscle fibers. Cellular exudate was not prominent, consisting mainly of small, scattered, perivascular collections of large mononuclear cells with occasional plasma cells and lymphocytes. There was moderate congestion and edema of the interstitial connective tissue. The significance of the appar-

ently minimal changes in these specimens is indicated by the heart in case 1, in which a perforation of the right atrium was present. Except near the site of perforation, where there was severe necrosis (Fig. 4), myocardial alterations were minimal.

In 4 hearts (cases 4, 5, 6, and 7) there was acute myocarditis of moderate severity. The predominant finding was the presence of cellular infiltrate, consisting of large mononuclear cells, situated usually around vessels and in the wider bands of interstitial connective tissue. Neutrophils were present in regions of more severe muscular degeneration (Fig. 5). In one heart a few multinucleated cells were seen. Degenerative changes were similar to those described in the preceding group.

In 6 hearts (cases 8, 9, 10, 11, 12, and 13) there was severe acute myocarditis. The most conspicuous findings were complete focal necrosis of muscle fibers and fairly abundant cellular infiltration (Figs. 6 to 12). Usually, severe necrosis of muscle involved only one or two adjacent muscle fibers. These muscle fibers were completely replaced by irregularly staining, disorganized masses of coagulated cytoplasm. Less marked degenerative changes, including vacuolation, fragmentation, and coalescence of the cytoplasm of muscle fibers, and karyolysis, were found to involve wide regions of myocardium. In the foci of severe muscular degeneration neutrophils usually were abundant. Fairly frequently, however, regions of severe necrosis were found with very little cellular infiltration in their immediate vicinity. In general, the cellular exudate was most prominent around the smaller vessels and in the wider bands of interstitial connective tissue. In these regions large mononuclear cells were numerous. Plasma cells and lymphocytes were less abundant. Congestion and edema of the interstitial connective tissue usually were conspicuous. Frequently the collagenous fibers of the interstitial connective tissue showed an increased affinity for eosin. There was straightening of collagen fibers and a tendency of fibers to form small clumps. This change was most marked around medium-sized vessels.

One heart (case 14) was classified separately as healed myocarditis. The outstanding change was the presence of large regions in the posterior wall of the left ventricle containing practically no muscle fibers (Figs. 13 and 14). In these regions the cellular components consisted mainly of large mononuclear cells situated in a rather delicate lacework of connective tissue fibers, suggesting that extensive complete necrosis and absorption of muscle fibers had occurred, leaving the myocardial stroma and a few macrophages. No vascular lesions were found in association. The remainder of the cardiac muscle showed minimal changes, including

vacuolation and hyaline droplet degeneration of cytoplasm, marked variations in cytoplasmic affinity for eosin, and karyolysis. There were a few scattered plasma cells and lymphocytes.

Selected hearts from each of the groups discussed previously, in which cytoplasmic vacuolation was present, showed only minimal deposition of lipid when stained with sudan III.

### *Endocarditis*

Minimal valvular changes were found almost constantly, with and without associated myocarditis. These changes included separation of valvular stroma as if by edema, and foci of highly eosinophilic bundles of collagen (Fig. 15). These findings were somewhat more marked in the aortic and mitral valves than in other valves and were most prominent in hearts showing myocardial changes.

As mentioned previously, in one patient (case 12) there was acute vegetative endocarditis of the mitral valve. The entire valvular stroma was infiltrated with numerous cells, mainly neutrophils and, to a lesser degree, lymphocytes (Fig. 16). The vegetations consisted of irregular deposits of fibrin which were undergoing organization (Fig. 17). No bacteria were seen. Within the valve leaflets there were scattered thin-walled vessels the size of arterioles, indicating previous vascularization of the valve and possibly, therefore, previous inflammation.

In one heart there was a subendothelial circumscribed mass, about 2 mm. in diameter, situated on the inferior aspect of the mitral valve. This lesion consisted of fibroblasts, budding capillaries, deposits of hemosiderin, and recent hemorrhage. Atypical for a healing vegetation, it may have represented a resolving hematoma.

The mural endocardium frequently showed changes in those hearts in which myocarditis was found. These changes included endothelial proliferation, subendothelial edema, and the presence of cellular infiltration, the cell types corresponding to those in the myocardium. There were no significant endocardial changes in those hearts not showing myocardial inflammation.

### *Endarteritis of a Patent Ductus Arteriosus*

As already mentioned, in one case (case 13) acute vegetative endarteritis of a patent ductus arteriosus was observed. The vegetations, consisting mainly of fibrin and leukocytes, were apparently of recent origin, although beginning organization was noted (Figs. 18 and 19). No bacteria were seen. There was moderately severe arteritis at the site of the vegetations, indicated by marked subintimal edema, many neutro-

phils, and slight endothelial proliferation. The left pulmonary artery was uninvolved. There was associated severe acute myocarditis (Fig. 20).

### *Pericarditis*

In one case (case 14) there were left pleuropericardial adhesions and obliterative pericarditis, raising the question of primary pleuritis with secondary pericarditis and myocarditis. Since there was only minimal inflammatory change in the epicardium, it did not seem likely that the pericarditis led to myocarditis. Although the epicardium in other hearts frequently showed inflammatory changes, in no instance were these changes sufficiently severe to indicate that pericarditis was primary.

### *Histologic Changes in Hearts Not Showing Myocarditis nor Endocarditis*

There were 21 hearts in which the histologic findings were not considered significant. All of these showed slight swelling of muscle fibers, and in most there was minimal vacuolation of cytoplasm with some congestion and separation of muscle fibers as if by edema. The latter findings are probably explanatory of the slight to moderate increase in heart weights shown in Table I.

### REPORT OF CASES

The following case reports are presented to illustrate certain unusual findings.

#### *Case 1*

A white boy, 7 years of age, living in a community in which there was poliomyelitis, was well until August 26, 1946, when he became febrile and vomited a few times. On August 29, headache and pains in his neck developed. He was admitted to the hospital on August 31 because of dizziness and difficulty in walking. On physical examination there was cervical rigidity but no definite evidence of paralysis. The blood pressure was 130/90 mm. of Hg. The cerebrospinal fluid contained 20 lymphocytes and 7 neutrophils per cmm. and the total concentration of protein was 65 mg. per 100 cc. Hot packs were applied to the neck and back, and penicillin was given. About 12 hours after admission, dysphagia, paralysis of the soft palate, and cyanosis developed. The patient was placed in a respirator, after which it was noted that the pulse was thready. Attacks of cyanosis continued but were partially relieved by the aspiration of secretions from the pharynx. On September 1, at about 12:45 a.m., the patient suddenly became cyanotic. The pulse rate rose from 100 to 180 per minute and the axillary temperature to 104.6° F. The patient died at 1:45 a.m. on September 1, 1946, 6 days after the onset of illness.

The anatomic findings in both the medulla and the spinal cord were characteristic of acute poliomyelitis.

The pericardial sac was found to be distended and grayish blue. When



it was opened, free blood was encountered, the estimated amount being about 100 cc. Hemopericardium was due to a perforation of the right atrium, located in its posterior wall to the right of the base of the valve of the coronary sinus (Fig. 1). The defect measured about 3 by 4 mm., was oval and had slightly roughened edges. There was recent hemorrhage into the surrounding epicardium.

The heart weighed 120 gm. Except for marked dilatation of the right atrium and moderate dilatation of the right ventricle, no significant gross cardiac abnormalities were noted. Histologic study showed mainly degenerative myocardial changes. Foci of swollen muscle fibers with loss of striations and fragmentation of individual muscle fibers were present in all sections examined. Near the perforation of the right atrium such areas were larger and the changes slightly more severe than at other sites (Fig. 4). Scattered neutrophils were present in the foci of more severe degenerative change.

There was bronchopneumonia confined to the lower lobe of the left lung. No significant changes were found in the other viscera.

### *Case 12*

A single white man, 34 years of age, had a familial history of diabetes but had been in good health. Following a poliomyelitis outbreak in his home state, on December 6, 1946, sore throat, anorexia, chills, and fever developed. On December 7 he noted difficulty in swallowing and speaking, and shortness of breath. He was admitted to the hospital on December 9. On physical examination cyanosis was evident and there was diminution of the deep reflexes of the upper extremities. Respirations were of a grunting character, preventing adequate cardiac examination. The blood pressure was 180/80 mm. of Hg; temperature, 102.6° F.; pulse, 120; respirations, 25. Leukocytes numbered 20,000 per cmm. of blood. The cerebrospinal fluid contained 45 lymphocytes and 5 neutrophils per cmm. and the total concentration of protein was 30 mg. per 100 cc. There was severe albuminuria, and a few hyaline and granular casts were found in the urine. The urine also showed a trace of reducing substance and the blood sugar was found to be 238 mg. per 100 cc. of blood. Fifteen thousand units of penicillin were given intramuscularly every 4 hours. Ten thousand units of diphtheria antitoxin were administered also. The patient was placed temporarily in a respirator but fought its use. On December 11, after the intravenous administration of a 10 per cent solution of glucose, cyanosis suddenly became more severe and the patient had a generalized convulsion. Use of the respirator was again instituted, but cyanosis continued and the patient died at 7:00 p.m. on December 11, 1946, 5 days after the onset of the illness.

The anatomic findings in both the medulla and the spinal cord were characteristic of acute poliomyelitis.

The heart weighed 340 gm. The right ventricle was slightly dilated. There were multiple, firm, translucent, grayish red vegetations, each measuring 1 to 2 mm. in diameter, near the free edge of the atrial surface of the mitral leaflets (Fig. 2). No gross evidence of old rheumatic

involvement was found. On histologic study, the vegetations were found to consist of fibrin with beginning invasion by fibroblasts (Fig. 17). No bacteria were seen in either the vegetations or the valvular stroma. The mitral leaflets showed marked separation of stromal elements as if by edema and there was extensive infiltration with cells, mainly neutrophils and, to a lesser degree, lymphocytes (Fig. 16). The valvular stroma contained thin-walled vessels the size of arterioles. There were several small masses of degenerating collagen in the tricuspid valve but the other valves did not present significant changes. The myocardium presented marked inflammatory changes, involving mainly the interventricular septum and the posterior papillary muscle. In these regions there were many foci of necrosis of muscle fibers. Fairly numerous cells, mainly neutrophils and large mononuclear cells, were found in the perivascular and interstitial connective tissue, and dense collections of neutrophils were present in the foci of severe muscle degeneration.

There was early bronchopneumonia. No embolic phenomena were observed. No significant changes were found in the other viscera.

### *Case 13*

A white girl, 7 years old, was well until August 2, 1946, when she complained of pains in her legs. Poliomyelitis was known to exist in her community. On August 3 difficulty in swallowing developed and she was admitted to the hospital. Physical examination showed palatal weakness but no other evidence of paralysis. The temperature was 100° F.; pulse, 80; respirations, 20. The cerebrospinal fluid contained 25 lymphocytes and 20 neutrophils per cmm. and the total concentration of protein was 50 mg. per 100 cc. The patient's condition remained apparently unchanged except for the development of tachycardia (140 per minute). At 6:00 p.m. on August 6, the patient rather suddenly went into a state of vascular collapse and, despite the intravenous administration of plasma, died at 1:06 a.m. on August 7, 1946, 5 days after the onset of the illness.

The anatomic findings in the spinal cord and medulla were characteristic of acute poliomyelitis.

The heart weighed 140 gm. The right ventricle was moderately dilated. No other significant gross cardiac abnormalities were noted. There was a small, irregular, brownish red, friable thrombus, about 3 mm. in diameter, projecting from the pulmonary ostium of a patent ductus arteriosus, the lumen of which measured about 2 mm. in diameter at the pulmonary end (Fig. 3).

Histologic examination of the myocardium showed foci of severe degeneration of muscle fibers in the right ventricle (Fig. 20). In these regions there were small groups of infiltrating cells, mainly neutrophils and large mononuclear cells. Other sections of the myocardium showed only minimal inflammatory changes.

The vegetative thrombus in the ductus arteriosus was apparently of very recent origin, consisting mainly of leukocytes held together loosely by fibrin. Beginning invasion by fibroblasts was noted along the area of attachment to the vessel wall (Figs. 18 and 19). No bacteria were seen in either the vegetation or the wall of the ductus. There was moderately severe superficial arteritis, indicated by subintimal edema, many neutrophils, and slight endothelial proliferation. The left pulmonary artery was uninvolved.

No embolic phenomena were demonstrated. Except for beginning bronchopneumonia, no significant changes were found in the other viscera.

### CORRELATION OF CLINICAL AND ANATOMIC FINDINGS

#### *Age*

The youngest patient found to have myocarditis was 7 years of age; the oldest was 44 years. The average age of the patients who had myocarditis was 23.0 years in contrast to the average of 15.3 years of the patients who did not have myocarditis. The average age of the patients who had severe or moderately severe myocarditis, acute or subacute, was 26.5 years, whereas the average age of the patients who had minimal myocarditis was 10.3 years. It appeared that older patients were more often subject to myocarditis than young patients and that the severity of myocarditis tended to be greater in proportion to age.

#### *Sex*

The over-all incidence of myocarditis was greater in male patients, with a ratio of about 2.5:1. Since there were, however, only 8 females in this series of 35 patients, the relative incidence of myocarditis was actually somewhat greater among females. It is of interest that both of the 2 pregnant women in this study (cases 7 and 10) were found to have myocarditis.

#### *Duration of Illness*

The patient with myocarditis who had the longest survival after onset of symptoms lived 57 days (case 14). The myocardial lesions in this case were classified as healed. The remaining 13 patients who had myocarditis lived less than 11 days after onset of symptoms, averaging 5.2 days of illness. This is only slightly more than half of the average length of illness (9.5 days) of the patients who did not have myocarditis, but since 2 of the patients without myocarditis had relatively long survivals (30 and 60 days, respectively) the importance of this comparison is questionable.

*Neurologic Findings*

Bulbar symptoms were present in 8 of the 14 patients who had myocarditis and in 14 of the 21 patients who did not have myocarditis. Five patients had severe, generalized paralysis, including symptoms of bulbar involvement. Three of these had myocarditis and 2 did not have myocarditis. Thus there seemed to be no correlation of either bulbar or severe generalized paralysis with the presence of myocarditis in this group of cases.

*Cardiac Findings*

Since respiratory distress in cases of poliomyelitis may result from involvement of either the spinal cord or the medulla, it was not surprising that dyspnea and cyanosis *per se* were found to be of little apparent value in the diagnosis of myocarditis. Among the 14 patients who had myocarditis, dyspnea had been recorded as a symptom in 12 and cyanosis in 11 patients. Among the 21 patients who were not found to have myocarditis, dyspnea had been noted in 18 and cyanosis in 13 patients. These findings do not, of course, exclude the possibility that a thorough clinical evaluation of the cardiac function of patients who have acute poliomyelitis, correlated with evidence of neurologic causes of respiratory distress, might disclose some diagnostic value for these symptoms.

Although the presence of pulmonary râles was frequently recorded, they could not be attributed definitely to cardiac failure, since it was impossible to exclude inflammatory congestion and the edema which frequently accompanies encephalitis. No peripheral edema was observed.

Precordial pain was present in only one patient. This patient was found not to have myocarditis.

The presence of cardiac murmurs was indicated in the clinical records of 3 patients, each of whom was found to have myocarditis (cases 3, 4, and 10). In each instance the murmur was at the base of the heart. In one patient it was transmitted to the cardiac apex. In another patient (case 7) the heart tones were muffled and the apex of the heart was displaced to the left. Since this patient was pregnant, the latter finding was of questionable significance. This patient was the only one in the series in whom severe bradycardia (44 per minute) was noted. Her case is one of those in the group with moderately severe myocarditis.

Tachycardia was commonly observed, but the incidence and severity of this finding were about the same in the group of patients without myocarditis as in the group with myocarditis. Irregularity of pulse was noted in 2 patients, only one of whom (case 4) had myocarditis.

Cardiac lesions were suspected clinically in 2 patients, one of whom

had a congenital fusion and fenestration of the cusps of the aortic valves but no myocarditis. The other patient (case 3) had myocarditis.

In none of the cases in this study was there a past history or other clinical evidence of rheumatic fever.

### *Suddenness of Death*

Six patients died rather suddenly, 3 of whom (cases 1, 10, and 14) were found to have myocarditis. Except in case 1, in which a perforation of the right atrium was found at necropsy, it was impossible to determine whether or not myocarditis had actually caused sudden death, since bulbar involvement with poliomyelitis was present in each case.

A general correlation of clinical and anatomic findings is presented in Table II.

### ETIOLOGY AND PATHOGENESIS

Before one ascribes the production of myocarditis to the virus of poliomyelitis it is necessary to consider, as has been indicated by Saphir, other possible etiologic factors, such as bronchopneumonia, serum reactions, and sulfonamide sensitivity, each of which has been reported to have caused myocarditis.

Bronchopneumonia was present in only 4 of the cases in which myocarditis was found, and in none of these patients was the pneumonia severe. It seemed unlikely that bronchopneumonia was of much significance in the pathogenesis of myocarditis.

Although myocarditis has been produced experimentally by the injection of horse serum and has even been reported in a few cases in which human beings have been treated with horse serum, this factor did not seem important as a cause of myocarditis in this group of cases. Only 3 patients who had myocarditis (cases 4, 9, and 12) had received horse serum, and in each the injections were begun 2 days or less before death. None showed clinical evidence of sensitivity to serum.

As has been shown both experimentally and clinically by French and Weller, sulfonamide compounds may cause myocarditis. In the present study 4 patients who had myocarditis (cases 7, 8, 10, and 14) had received a sulfonamide compound, in each instance sulfadiazine. Three of these were given the drug for 2 days or less. There was no associated clinical nor other histologic evidence of toxicity. Administration of sulfonamide compounds seemed of minimal importance as a cause of myocarditis in this series of cases.

The effects of anoxia and general toxemia were impossible to assess adequately. It is probable that these factors may have caused at least some of the minimal cardiac changes observed in the cases in this study.

The reported occurrence of myocarditis in other virus diseases, in-

TABLE II  
Correlation of Clinical and Anatomic Findings in 14  
Patients Who Had Significant Cardiac Lesions

Case	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Age, years	7	9	15	21	22	26	36	21	31	32	44	34	7	17
Sex	M	M	M	M	M	M	F	F	M	F	M	M	F	M
Approximate duration of illness, days														
Type of paralysis	6 Bulbar	6 Bulbo-spinal	7 Spinal	3 Spinal	4 Spinal	10 Bulbar	4 Bulbar	3 Spinal	6 Spinal	5 Bulbo-spinal	4 Bulbar	5 Bulbo-spinal	5 Bulbar	57 Spinal
Dyspnea*	3	2	3	2	3	3		3	2	3		3		3
Cyanosis*	3	3	3		3	3	2	2	3	3		3		3
Oral temperature, °F.†	103	101	102	100.5	102.5	101	102	103	102.5	101	102	101	100	100
Pulse rate, beats per min.†	130	90	120	120	110	80	125	110	80	95	100	110	120	120
Respiratory rate, per min.†	35	33	20	36		20	30	22	30	30		20	30	20
Cardiac murmurs			Basal and apical system	Pulmonary system					Basal system					
Leukocyte count, per cmm. of blood		20,600	9,900			10,600	9,800	11,400	22,700				20,500	14,600
Myocarditis*	1†	1	1	2	2	2	2	3	3	3	3	3	3	3
Valvulitis*														
Endarteritis of a patent ductus arteriosus*														
Pneumonia*	2								1			1	2	1

\* Severity on a basis of 1 to 4.

† Approximate average during period of hospitalization.

‡ Even though myocarditis was minimal in case 1, there was a perforation of the right atrium.

cluding mumps, virus influenza, and epidemic encephalitis, suggests that it is not illogical to consider the virus of poliomyelitis to be capable of causing myocarditis.

Recently Helwig and Schmidt have succeeded in isolating a virus with both neurotropic and cardiotropic features. When injected into mice this virus was shown to cause paralysis of the extremities and myocarditis. These authors did not state the immunologic relationships, if any, of this virus to the virus of poliomyelitis, but this report of a virus disease with features grossly resembling poliomyelitis and with associated myocardial lesions again suggests that it is not unreasonable to suspect the poliomyelitic virus of causing myocarditis.

Since myocarditis was present in 40 per cent of the cases in this series and since other investigators have found an even greater incidence of myocarditis in acute poliomyelitis, it is evident that myocarditis occurs frequently in poliomyelitis. Inasmuch as it has not been possible to explain satisfactorily the etiology of myocarditis in the cases in this series without ascribing it to the virus of poliomyelitis, we are led to the assumption that the virus may invade and destroy cardiac muscle.

The occurrence of endocarditis and ductal endarteritis, such as was observed in cases 12 and 13, is more difficult to relate to the virus of poliomyelitis. Certain proof of the exact etiology of lesions of this type occurring in poliomyelitis, as well as proof of the cause of myocarditis, will depend on the isolation of the virus from such lesions and the experimental production of similar changes.

If it is assumed that the virus of poliomyelitis causes cardiovascular lesions, we are confronted with the problem of the mode of invasion of cardiovascular structures by the virus. It may be of some significance that the virus has been demonstrated in the blood stream of monkeys with poliomyelitis. On the other hand, most investigators believe that the virus migrates along nerves. Evidence of injury to nerves was observed in many of our cases and Saphir and Wile pointed out that nerve fibers of the myocardium were found to be separated by an "edema-like material." Further study will be necessary to evaluate such changes.

It is of interest that cases in which myocarditis was found tended to occur in chronologic sequence. This fact suggests that the poliomyelitis virus manifests marked cardiotropic features in only certain epidemics. This may explain why competent observers frequently have not found evidence of myocarditis.

#### COMMENT

Since acute myocarditis is seldom characterized by histologic features which will permit an etiologic diagnosis, it was not surprising that myo-

carditis in this series of cases presented a nonspecific picture. The lesions observed were similar to those which have been described in other infectious diseases and in Fiedler's myocarditis. No Aschoff bodies were found, although there was some degeneration of collagen such as has been described in rheumatic myocarditis. This finding seemed of minimal importance since degeneration of the collagen is rather frequent in the presence of inflammation, regardless of its cause.

It is unfortunate that blood cultures were not made in the 2 cases in which vegetative lesions were found, but the absence of demonstrable bacteria in these lesions is negative evidence in favor of infection by poliomyelitis virus. It is of interest that the lesions in both of these cases involved regions in which there was probably diminished resistance to infection—in one case a patent ductus arteriosus, in the other case a valve which was vascularized, indicating possible previous valvular injury. The absence of such *loci minoris resistentiae* in most patients who have poliomyelitis may partially explain why so few lesions of this character are observed.

The failure of physicians to establish the clinical diagnosis of myocarditis in cases of acute poliomyelitis is not difficult to understand. Myocarditis other than that occurring in rheumatic fever is almost always extremely difficult to diagnose. Moreover, serious neurologic symptoms in acute poliomyelitis have diverted clinical attention from the cardiovascular system. Perhaps a general awareness of the frequency of myocarditis in acute poliomyelitis will facilitate the clinical diagnosis of myocarditis.

#### CONCLUSIONS

Myocarditis occurs frequently in acute poliomyelitis, having been observed in 14 (40.0 per cent) of 35 cases of fatal poliomyelitis.

Acute vegetative endocarditis and endarteritis of a patent ductus arteriosus may occasionally be found in acute poliomyelitis. One example of each of these lesions was present among the cases in this study.

Since the cardiovascular lesions occurring in the cases included in this study were otherwise unsatisfactorily explained, poliomyelitis virus must be considered as a possible cause of such lesions.

Proof that cardiovascular lesions in acute poliomyelitis are caused by the poliomyelitis virus will depend on demonstration of the virus in the lesions and the experimental production of such lesions.

The diagnosis of myocarditis in acute poliomyelitis is seldom made during life. Myocarditis should be suspected in every patient who is seriously ill with acute poliomyelitis.

Myocarditis, as observed in this series of cases, was usually more



severe and proportionately more common in adults than in young children.

The ratio of males to females in the group of patients with myocarditis was 2.5:1, but since the ratio of males to females in the study was more than 3:1, actually a slightly greater proportion of females had myocarditis.

There was no specific correlation of type of paralysis—bulbar or spinal—with the presence or absence of myocarditis.

The actual rôle of myocarditis as a cause of sudden death in acute poliomyelitis could not be determined in this study, except in one patient, who had a perforation of the right atrium. Three of the 6 patients who died suddenly were found to have myocarditis, but all of these patients had bulbar involvement, which might have explained their sudden deaths.

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[ Illustrations follow ]

## DESCRIPTION OF PLATES

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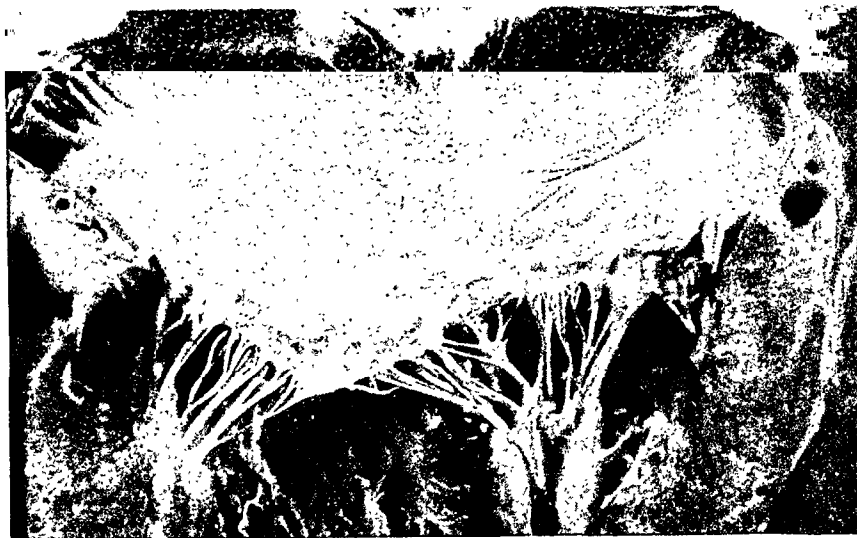
### PLATE 48

- FIG. 1. Case 1. Perforation of the posterior wall of the right atrium. The defect is located just to the right of the ostium of the coronary sinus.
- FIG. 2. Case 12. Left atrium and ventricle showing vegetations along the line of closure of the mitral valve. The absence of gross evidence of old rheumatic involvement is apparent.
- FIG. 3. Case 13. Portion of left pulmonary artery with aortic arch and descending aorta in background. Small vegetative thrombus may be seen at pulmonary ostium of a patent ductus arteriosus.
- FIG. 4. Case 1. Right atrium near site of perforation shown in Figure 1. Myocardial fibers have become granular and coalescent. A few neutrophils have infiltrated the area. Hematoxylin and eosin stain.  $\times 580$ .
- FIG. 5. Case 5. Myocardium showing abundant interstitial collections of neutrophils and large mononuclear cells with degenerative alterations of myocardial fibers. Hematoxylin and eosin stain.  $\times 160$ .
- FIG. 6. Case 10. Myocardium with focus of severe degeneration and infiltration of neutrophils. Hematoxylin and eosin stain.  $\times 190$ .

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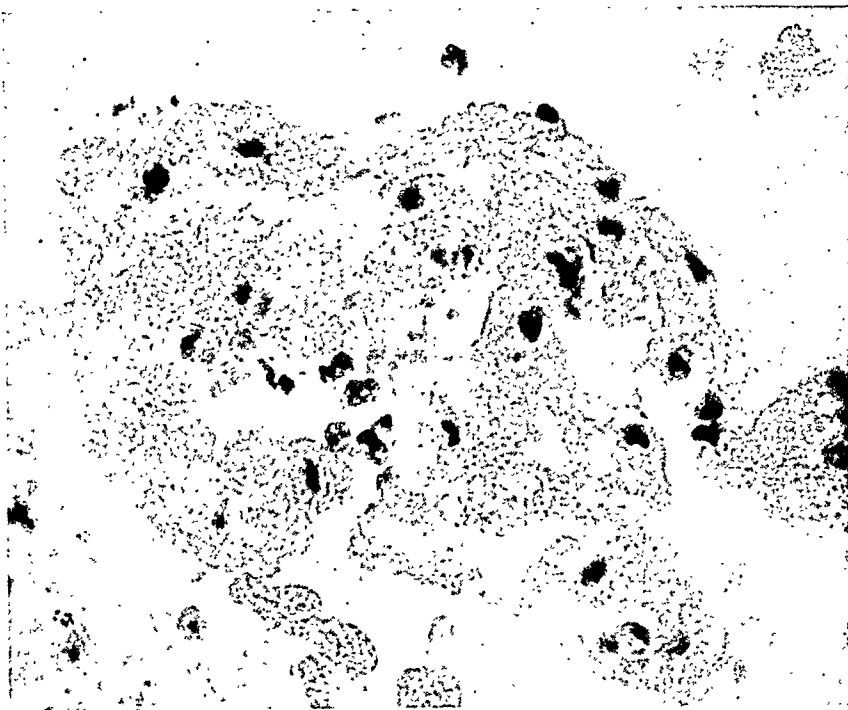
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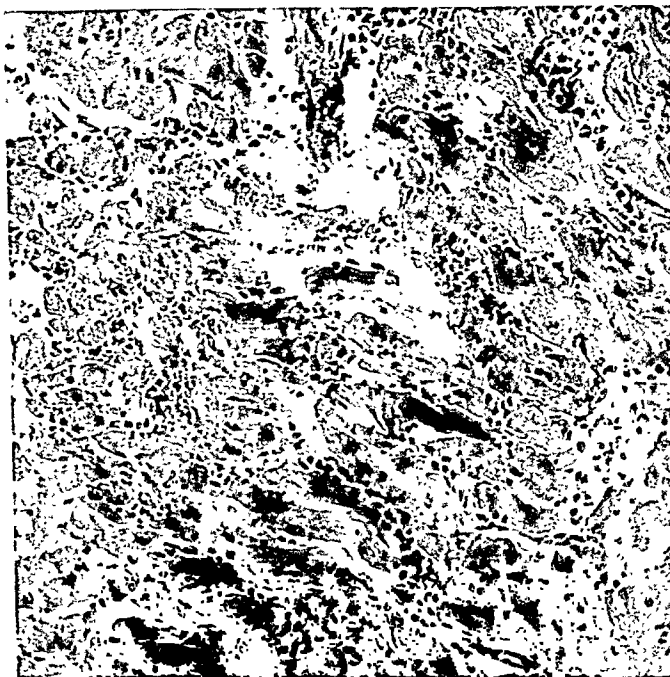
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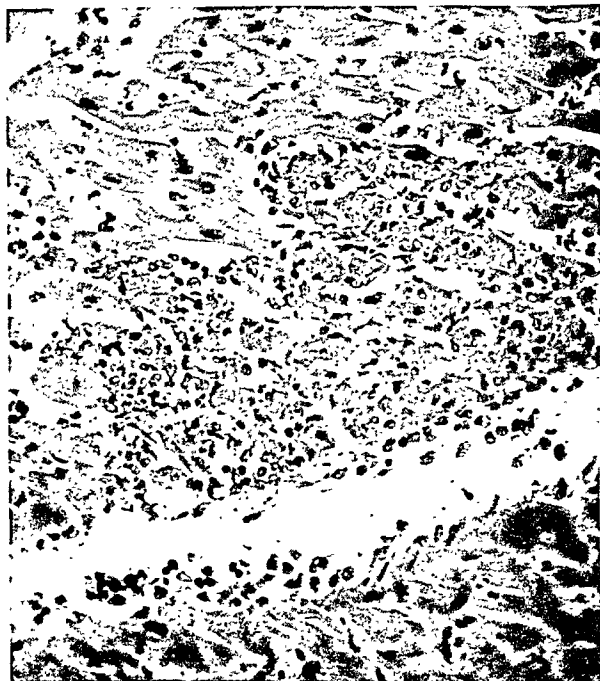


PLATE 49

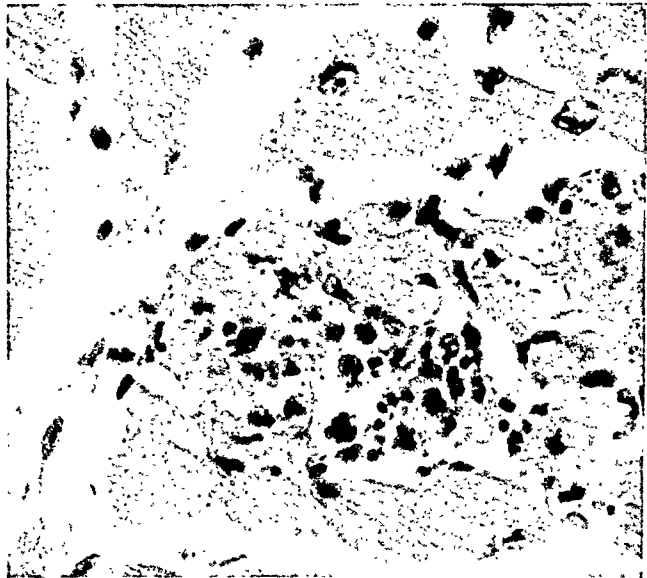
FIGS. 7 to 12. The apparent manner of progression of myocardial lesions is illustrated in this series of photomicrographs. Characteristic coagulation necrosis of myocardial fibers is well shown in *Figures 7 and 8* (case 8), stained with Mallory's phosphotungstic acid stain. In *Figures 9 and 10* (case 9) and in *Figure 11* (case 11) infiltration of neutrophils into areas of myocardial necrosis appears. In *Figure 12* (case 12) there is a small area of absence of myocardial fibers. Such a sequence of events probably explains the lesion seen in *Figure 13*. (Figures 7 and 8 were stained with Mallory's phosphotungstic acid stain; Figures 9 to 12 with hematoxylin and eosin. Figure 7,  $\times 880$ ; Figure 8,  $\times 800$ ; Figure 9,  $\times 1275$ ; Figure 10,  $\times 435$ ; Figure 11,  $\times 600$ ; and Figure 12,  $\times 450$ .)

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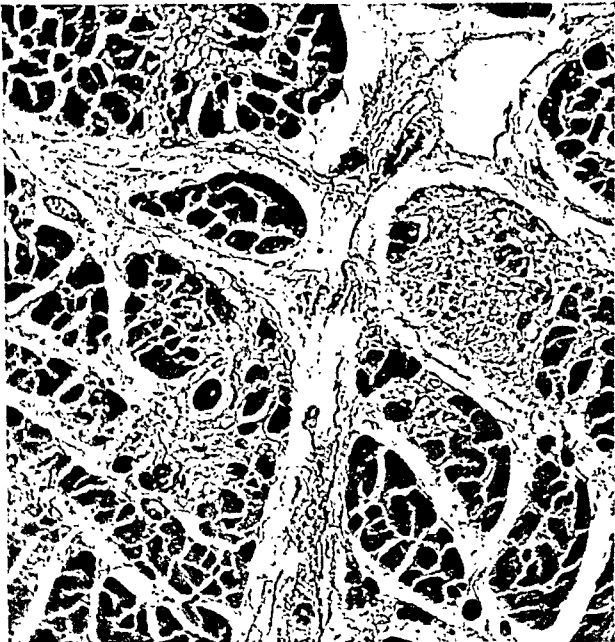
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Carditis in Poliomyelitis

## PLATE 50

- FIG. 13. Case 14. Posterior wall of left ventricle. Healed myocarditis, apparently representing the end-result of resolution of an acute process, as shown in Figures 7 to 12. Myocardial fibers have disappeared, leaving only stroma. Hematoxylin and eosin stain.  $\times 85$ .
- FIG. 14. Case 14. Higher magnification of a lesion shown in Figure 13. Lace-like pattern of remaining myocardial stroma is evident. Hematoxylin and eosin stain.  $\times 295$ .
- FIG. 15. Case 8. Mitral valve. Edema of valvular stroma and thickening of collagen fibers, such as were commonly observed with and without associated myocarditis. Hematoxylin and eosin stain.  $\times 260$ .
- FIGS. 16 and 17. Case 12. Mitral valve. In Figure 16 is seen infiltration of valvular stroma with neutrophils. Figure 17 shows an organizing fibrinous vegetation. Hematoxylin and eosin stain. Figure 16,  $\times 800$ ; Figure 17,  $\times 275$ .
- FIG. 18. Case 13. Low-power magnification of a section of the junction of the patent ductus arteriosus and the left pulmonary artery. The lumen of the left pulmonary artery occupies the upper portion of the field. The pulmonary ostium of the patent ductus arteriosus is occluded by a thrombus. Verhoeff's elastic tissue stain counterstained with van Gieson's connective tissue stain.  $\times 11$ .

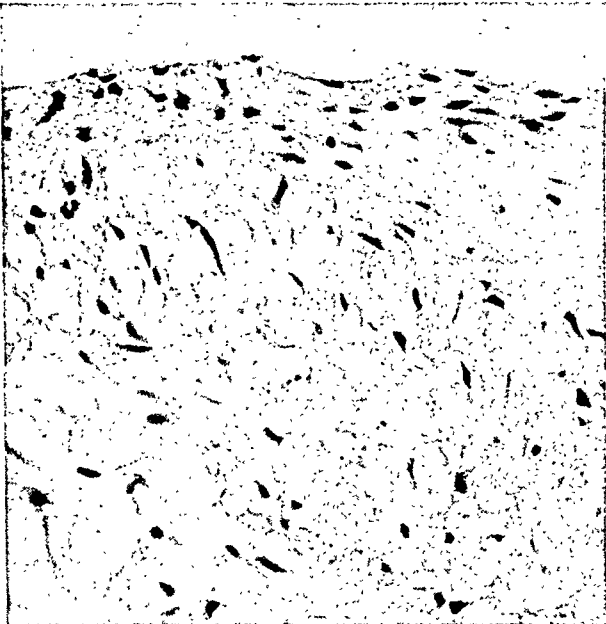
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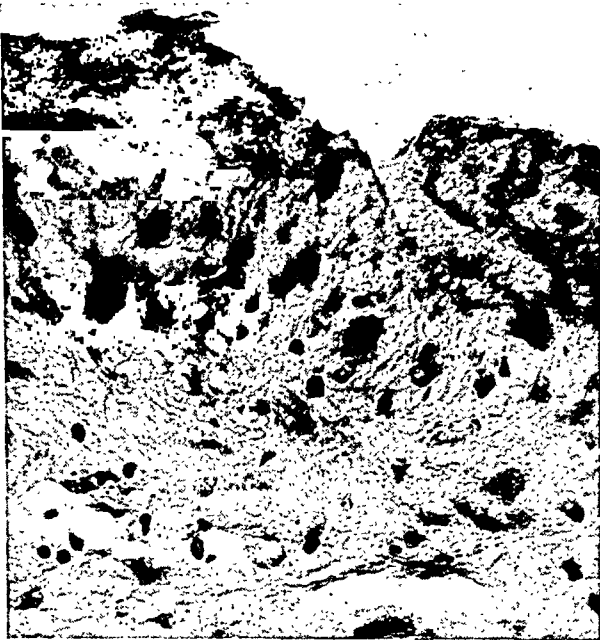
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PLATE 51

FIG. 19. Case 13. Recently formed thrombus and acute endarteritis of patent ductus arteriosus. Subintimal collection of neutrophils. Hematoxylin and eosin stain.  $\times 320$ .

FIG. 20. Case 13. Severe acute myocarditis. Focal necrosis of myocardial fibers and infiltration of neutrophils. Hematoxylin and eosin stain.  $\times 200$ .

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Carditis in Poliomyelitis



# THE FATE OF BLOOD INJECTED INTO THE ARTERIAL WALL \*

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The experiments which are described in this paper were undertaken with the purpose of determining whether hemorrhage in the intima or media of an artery plays a part in the initiation of arteriosclerosis or of dissecting aneurysm. That the intima and media often are richly vascularized in arteriosclerosis and idiopathic medial necrosis has been known for many years, but recently Leary,<sup>1</sup> Nelson,<sup>2</sup> Paterson,<sup>3</sup> Wartman,<sup>4</sup> and Winternitz, Thomas, and Le Compte<sup>5</sup> have shown that hemorrhage from such vascular plexuses may lead to the formation of large hematomas, or of dissecting aneurysms. Such intramural hemorrhages are important because they may obstruct the lumen by their size, or they may precipitate thrombosis. Since surprisingly little is known about what happens to these intramural hemorrhages, it seemed desirable to try to obtain such information experimentally.

## METHODS

Healthy, adult bitches were used in all experiments. They were fed purina chow diet and given as much water as they would drink. The common carotid and femoral arteries and the abdominal aorta were exposed with as little trauma as possible, and homologous blood, which was obtained either from the same artery or from an adjacent vein, was injected through a hypodermic needle into the arterial wall. Nothing was added to the blood and the injection was accomplished before clotting occurred. Multiple injections were made in the arteries for a distance of several centimeters. The sites of injection were marked by silk sutures placed in the neighboring tissues. All surgical operations were performed aseptically under nembutal anesthesia.

Later, the arteries were removed at intervals of a few days to many months so that the hematomas could be studied at various ages. Immediately upon removal the involved segment of artery was placed in 10 per cent neutral formalin for 24 hours and then cleared by Spalteholz' method. The areas of hemorrhage were identified easily in the cleared specimens, and blocks were cut of the affected portions and embedded in paraffin. Sections were cut at  $4\mu$  and stained with hematoxylin and eosin, Masson's trichrome stain, and a combination of Weigert's elastica and

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van Gieson's collagen stains. The Perls and Turnbull tests were used to demonstrate iron pigment.

## RESULTS

It was found that the blood was situated in either the media or the adventitia of the artery but that there was none in the intima. This probably was because the normal intima of dog arteries is extremely thin so that it is virtually impossible to inject significant quantities of blood into it. Thus the results concern principally medial hemorrhages and afford little information about intimal hemorrhages. The effects of the trauma associated with the surgical operations and with the actual injection of the blood could not be separated from the effects caused by the presence of the blood alone.

The results were the same when blood was injected into the carotid arteries, the femoral arteries, or the abdominal aorta; nor were they different in the case of arterial or venous blood. Blood previously withdrawn from the recipient animal was used in all experiments. The resulting hematomas varied in size from 1 to 5 mm.

The changes which were observed in the media will be described briefly, the details being given in Table I.

Eight dogs were used and a total of 26 hematomas were produced in 18 arteries. The hematomas were removed for histologic examination as follows: 1 after 3 days; 4 after 8 days; 1 after 15 days; 1 after 24 days; 1 after 48 days; 5 after 61 days; 1 after 103 days; 4 after 169 days, and 8 after 392 days.

By the third day, organization of the hematoma was already evident and there were many fibroblasts and a few immature capillaries. Nevertheless, the erythrocytes were still well preserved, there was no visible pigment, and the iron stains were negative. Fibrin and moderate numbers of neutrophils were present. There was no demonstrable change in the intima, but the media showed chromotropic degeneration and fragmentation of muscle fibers.

After 8 days the red blood cells were still numerous and appeared well preserved, but pigment was present. This was in two forms. One form was dark yellowish brown, finely or coarsely granular, and stained positively for iron. The other form was very bright yellow, homogenous, and often was precipitated on the elastic fibers. It did not stain positively for iron. Organization of the hematoma had progressed and there were giant cells which had phagocytized some of the iron-containing pigment as well as cellular debris. Fibrin and neutrophils still were present. One of the four arteries observed at this stage showed a hyaline intimal thrombus directly over the hematoma (Fig. 1).

Distinct, well formed capillaries filled with blood first were observed in a hematoma at the end of 15 days (Fig. 3). Despite the presence of these capillaries and an increased amount of iron-containing and non-iron-containing pigment, most of the hematoma was still composed of

TABLE I  
*Summary of Findings Following Injection of Blood into Arterial Walls*

Dog	Artery injected	Age of hematoma (days)	Red blood cells	Iron pigment	Medial scar	Vascularization of media	Intimal plaque
838	Abdominal aorta	3	+++	o	o	o	+
789	Right femoral	8	+++	o	Necrosis	o	o
789	Left femoral	8	+++	++	Necrosis	o	++
							Thrombus
873-A	Left carotid	8	+++	++	Necrosis	o	o
873-B	Left carotid	8	+++	++	o	o	o
808	Right carotid	15	+++	++	o	++	o
838	Right femoral	24	o	+++	o	+	++
1380	Right carotid	48	o	o	+	+	o
1379-1	Left carotid	61	o	+++	++	++	o
1379-2	Left carotid	61	o	+	+++	+++	o
1379-3	Right carotid	61	o	++	++	++	o
1379-4	Right carotid	61	o	+++	++	++	o
1379-5	Right carotid	61	o	o	++	+	o
981	Left femoral	103	o	o	+++	o	++
1062	Right carotid	169	o	o	+	o	o
1062	Left femoral	169	o	o	+	o	o
1062	Left carotid	169	o	o	o	o	o
1062	Right femoral	169	o	o	o	o	o
1378-1	Left femoral	392	o	o	++	o	o
1378-2	Right femoral	392	o	o	+++	o	+
1378-3	Right femoral	392	o	o	++	o	+
1378-4	Left carotid	392	o	o	+++	o	o
1378-5	Left carotid	392	o	o	+	o	o
1378-6	Right carotid	392	o	++	+	o	o
1378-7	Right carotid	392	o	o	+++	+	++
1378-8	Right carotid	392	o	+	++	+	o

well preserved red blood cells (Fig. 2). Fibrosis of the hematoma had not occurred. Otherwise the findings were the same as in the arteries examined at the end of 8 days.

After 24 days the red blood cells had completely disappeared and there was a large amount of pigment, most of which stained specifically for iron. Immature fibrous connective tissue as well as fibroblasts and a few capillaries accompanied by mononuclear cells and giant cells were present. No neutrophils or fibrin were observed. One artery showed thickening of the intima over the hematoma by fibroblasts.

The changes were much the same after 48 days, except that there was distinct scarring of the media. Mature collagenous connective tissue, which was well supplied with capillaries, was present.

Vascularized medial scars were still present at the end of 61 days

(Fig. 4). As far as could be determined, the walls of the capillaries were intact and there was no indication that hemorrhage had occurred from any of them (Fig. 5). Small amounts of iron-containing pigment, presumably from the original hematoma, were present. There was no cellular exudate. Occasionally the intima was slightly thickened by the presence of elastic fibers.

The single hematoma which was available for examination at 103 days showed a large, richly vascularized scar in the media and focal fibrosis of the overlying intima (Fig. 6). Neither red blood cells nor pigment were present.

Of 4 arteries observed after 169 days, none showed either red blood cells or pigment. In 2 the vessel walls were restored to normal and only the silk marker remained to indicate where the blood had been placed. In the other 2 vessels there were avascular medial scars.

Eight hematomas were examined after 392 days. Of these, 2 showed complete restitution of the vessel wall so that no evidence of the previous hematoma remained. Medial scars were found in 6 vessels, in 2 of which iron pigment still was present, and in one of which there was a small amount of calcium (Fig. 9). Four of the scars were avascular (Fig. 7) while 2 contained a few capillaries. There was no evidence of hemorrhage from these capillaries. Thickening of the intima over the scar was observed in 4 instances, resulting from proliferation of endothelial cells, collagen, and elastica (Fig. 8).

## DISCUSSION

It should be pointed out that neither the formation of atheromas nor dissecting aneurysm was observed in these experiments, and that the new capillaries which grew in the medial hematoma did not bleed. Intimal thrombosis was observed in only 1 of the 18 arteries which were examined and this was but a small platelet and fibrin thrombus. The whole process appeared to be one of organization of the hematoma resulting either in a medial scar or in restitution of the arterial wall. The lesion apparently was self-limited and did not progress to either arteriosclerosis or dissecting aneurysm.

Removal of the blood from the arterial media was accomplished by gradual destruction of red blood cells accompanied by liberation of pigment and of fluid and cellular exudate in which neutrophils predominated. Medial necrosis was present for the first 8 days but was not observed after that time. Red blood cells disappeared between the 15th and 24th days. Hemosiderin pigment was observed first after 8 days and in most instances continued for as long as 61 days. Iron-containing pig-

ment was absent in 4 vessels examined at the end of 169 days, but was discovered in 2 of 8 arteries examined after 392 days. Scars were first seen in the media at the end of 48 days. They were composed of collagenous connective tissue and contained capillaries which tended to disappear between 103 and 169 days, although in 2 animals they were observed after 392 days. Macrophages containing sudanotropic material were not seen at any time.

The significance of the intimal sclerosis which was observed is not clear since a similar lesion was observed in arteries which had not been injected with blood, as well as in untreated control animals (Fig. 10). The sclerotic lesions contained collagenous connective tissue and elastic fibers and sometimes were partly hyalinized, but they were never vascularized nor was atheromatous degeneration observed. Intimal sclerosis was observed 7 times: in one hematoma each after 3, 8, 24, and 103 days, and in 3 hematomas after 392 days.

The occurrence of capillaries in the hematomas is of considerable interest because of the frequency with which they are discovered in the diseased arteries of man. In these experiments they were observed most commonly from the 15th to the 61st day and only a few as late as 392 days, suggesting that the capillaries tended to disappear as the hematoma healed, leaving an avascular scar. They contained blood and always were well formed, with a distinct endothelial lining. They never were observed to rupture and bleed, and thus small hemorrhages such as occur so frequently around the capillaries in an arteriosclerotic artery were completely absent. The capillaries were seen to arise from the vasa vasorum, but in no instance, even though serial sections were examined, were they discovered to communicate with the lumen of the artery, which is another point of difference between the experimentally produced capillaries in normal dog arteries and the naturally occurring capillaries in diseased human arteries.

#### SUMMARY

Homologous blood was injected into the media of the common carotid and femoral arteries and of the abdominal aorta of normal dogs. Under the conditions of the experiment this blood disappeared within 2 months and the artery either healed, leaving no histologic evidence of the hematoma, or a scar formed in the media. These medial scars were observed as early as 48 days and as late as 392 days after the blood was injected. All of the younger scars were well vascularized with capillaries, but most of the older ones were avascular. The hemorrhages did not enlarge and dissecting aneurysm was not produced. Medial necrosis was seen early but not after 15 days. Although intimal sclerosis was dis-



covered in association with nearly 30 per cent of the hematomas, atheroma did not develop. This intimal sclerosis could not be distinguished from histologically similar lesions which occurred naturally in uninjected arteries and in control animals.

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#### DESCRIPTION OF PLATES

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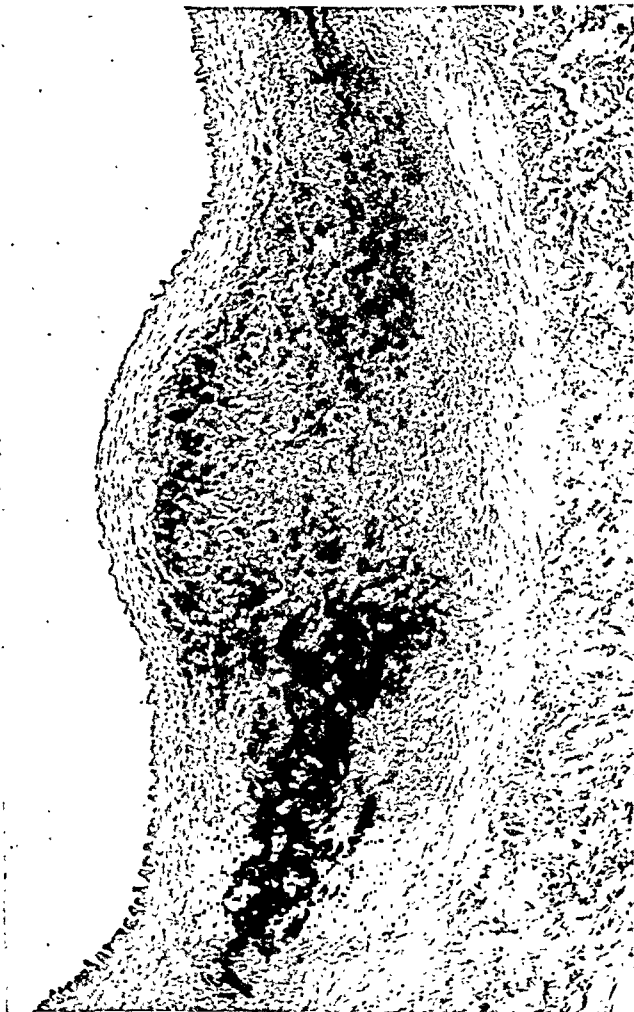
##### PLATE 52

- FIG. 1. Dog 789. Fibrin and platelet thrombus covering the intima over the site of injection of blood 8 days after operation. This was the only case in which thrombosis was observed. Hematoxylin and eosin stain.
- FIG. 2. Dog 808. Medial hematoma observed 15 days after injection of blood into the right common carotid artery. The wavy black bands at the lateral margins of the hematoma are deposits of blood pigment. Hematoxylin and eosin stain.
- FIG. 3. Dog 808. Higher magnification of a portion of Figure 2 showing a well formed capillary. Hematoxylin and eosin stain.

1



2



3



PLATE 53

- FIG. 4. Dog 1379-2. A richly vascularized medial scar discovered 61 days after the injection of blood into the media of the left common carotid artery. The intima is normal. Hematoxylin and eosin stain.
- FIG. 5. Dog 1379-3. Capillaries in the medial scar of the right common carotid artery 61 days after injection of blood. The intima is normal. Hematoxylin and eosin stain.
- FIG. 6. Dog 981. Thickening, fibrosis, and hyalinization of the intima and scarring of the media 103 days after injection of blood into the left femoral artery. There is no atheroma and the internal elastic lamina is intact. Hematoxylin and eosin stain.

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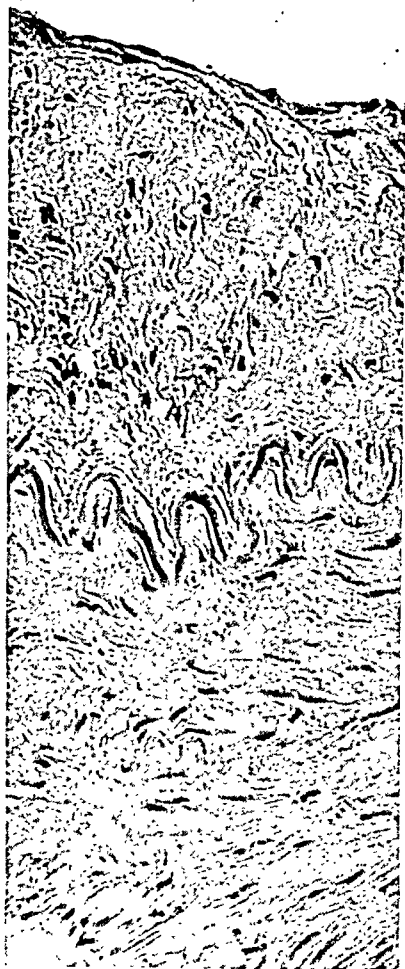
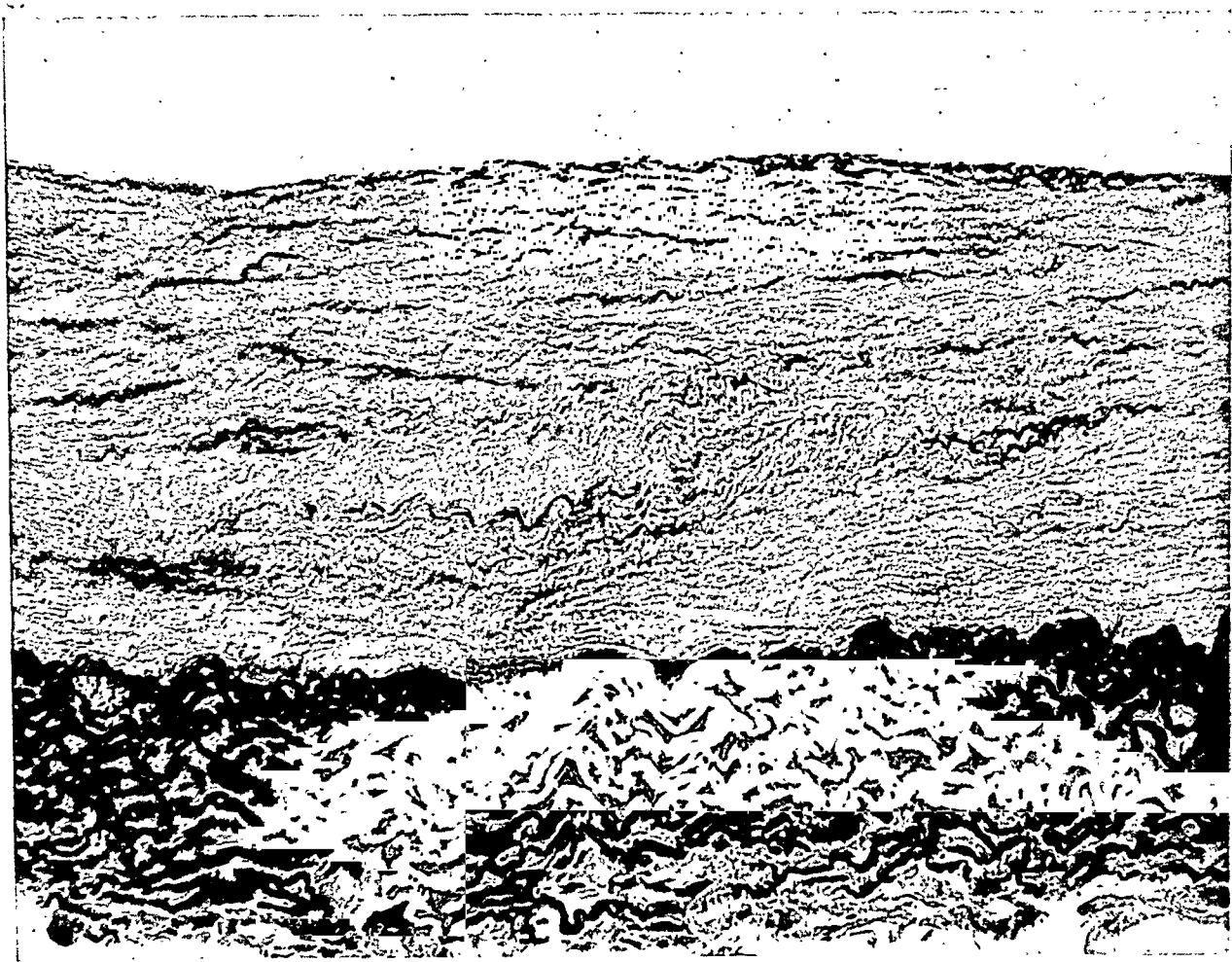


PLATE 54

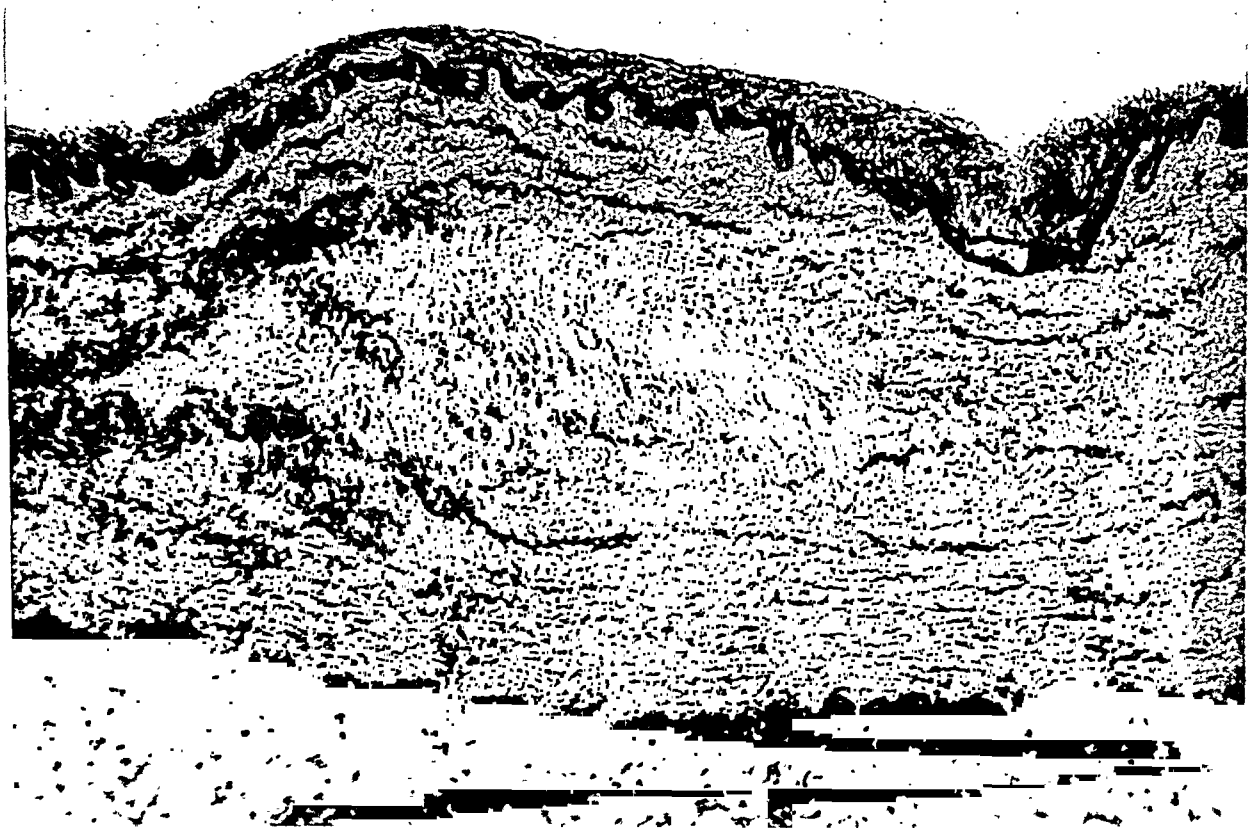
FIG. 7. Dog 1378-1. Small avascular medial scar 392 days after injection of blood into the left femoral artery. Van Gieson's and elastica stains.

FIG. 8. Dog 1378-7. Section of right common carotid artery 392 days after injection of blood into wall, showing a medial scar with a few mature capillaries, and intimal fibrosis. Van Gieson's and elastica stains.

7



8



Wartman and Laipply

Blood Injected into Arterial Wall

PLATE 55

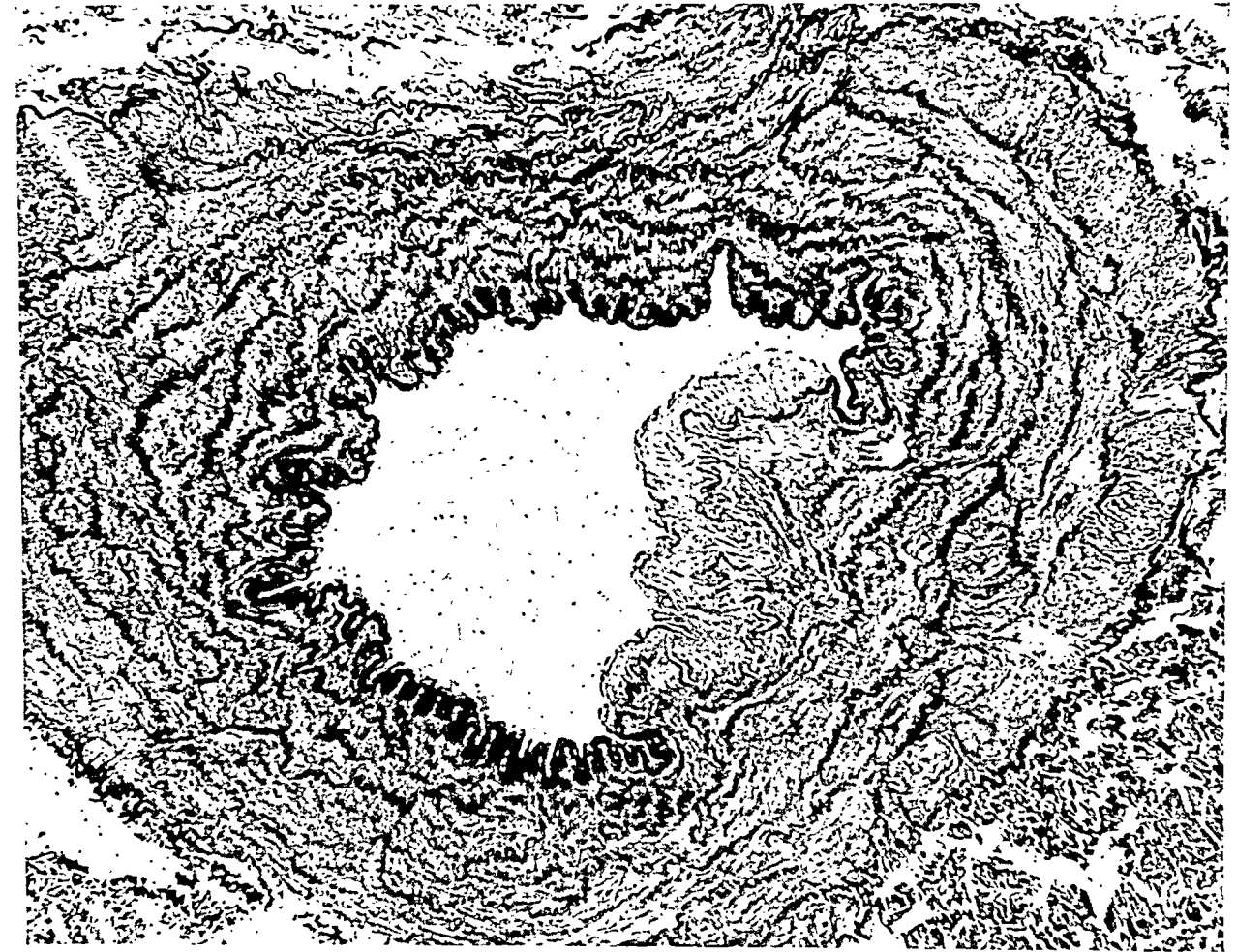
FIG. 9. Dog 1378. Avascular medial scar with small deposit of calcium 392 days after injection of blood. Hematoxylin and eosin stain.

FIG. 10. Dog 1379. A small artery showing spontaneous intimal sclerosis. No blood was injected into this artery nor was it manipulated. This is thought to be the canine counterpart of human arteriosclerosis, but differs in that atheromatous degeneration usually does not occur. Van Gieson's and elastica stains.

9



10







# GASTRIC SUBMUCOSAL GRANULOMA WITH EOSINOPHILIC INFILTRATION \*

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In view of the great interest aroused by recent work on the so-called eosinophilic or histiocyto-eosinophilic granuloma (Beck, 1943, Škorpil, 1946), I am presenting several cases showing in the submucosa of the gastric antrum granulation tissue of a peculiar type associated with eosinophilic infiltration.

## REPORT OF CASES

### *Case 1*

K. E., a male, 42 years old, had been suffering from "stomach trouble" for some time. He had lost weight and had symptoms pointing to pyloric stenosis. The gastric juice showed normal hydrochloric acid values. Roentgenograms showed pyloric stenosis with a thickening of the mucosa and some gastric retention. Gastric resection (Péan-Rydygier) was performed.

The surgical specimen (no. 6584/44) consisted of the pyloric part of the stomach and was 9 cm. in length. The mucous membrane showed no defect or scar and was freely movable. In the submucosa near the pylorus there was a soft, almost transparent, ill defined node, the size of a small plum, covered with normal mucous membrane. On section it was grayish white to yellow and the cut surface presented many small openings.

Microscopically, the mucous membrane showed atrophic gastritis with islands of metaplastic glands of intestinal type. There was considerable lymphocytic and also a moderate eosinophilic infiltration of the stroma. The lymphatic glands of the lesser curvature showed some hyperplasia.

The node in the submucosa of the pyloric part consisted of cells of the following types (Fig. 5): (1) Fusiform cells with ill defined plasma and oval nuclei poor in chromatin, obviously fibroblasts. Some of these cells were star-shaped. Between the cells a loose network of collagenous fibers could be seen. (2) Irregularly distributed lymphocytes, occasionally accumulated to form rudimentary lymph follicles. (3) Numerous eosinophilic cells of polymorphonuclear type, distributed evenly throughout the pathologic tissue.

The arterioles and capillaries were rather numerous. In addition there were small, round cavities lined with endothelium, obviously corresponding to the openings seen on gross examination. Some of them

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appeared empty while others were filled with a homogenous or foamy substance staining pink with eosin and containing a few lymphocytes. The larger cavities were surrounded by loose fibrous tissue. Undoubtedly all of these cavities were dilated lymphatic vessels.

The relation of the node to the neighboring structures was of interest. The muscularis mucosae was well preserved, in part, separating the abnormal tissue from the mucosa. In places, however, the tissue penetrated between its fibers, and at some points it reached the mucosa. Toward the duodenum it could be traced between the glands of Brunner. In the opposite direction it stopped in the submucosa. Everywhere its borders were ill defined, lacking any tendency to encapsulation. The mucous membrane covering this particular tissue was somewhat thinned out.

### *Case 2*

O. Z., a woman, 64 years of age, had been complaining for about 6 months of pain below the processus xiphoideus, especially during meals. The pain was persistent, not accompanied by belching, and disappeared at rest. There was no hunger pain at night. Vomiting occurred once only, just after a meal. The patient lost 10 kg. of weight. She had not been treated previously. On palpation no resistance or tenderness of the epigastric area was found. Roentgenograms revealed a tumor of the prepyloric part of the stomach. At operation the stomach was found to be freely movable and small. In the prepyloric part a soft, elastic resistance the size of a plum was felt. Resection according to the method of Péan-Rydygier was performed and the patient left the hospital 19 days later in good condition.

In the resected stomach (no. 3991/45), about 2 cm. above the pylorus, there was found a polypoid growth, 2.7 by 1.6 by 1.4 cm. (Fig. 1). It was soft and elastic, and its smooth surface had a brownish violet color. The stalk was 1 cm. long and as thick as a quill.

Microscopically (Figs. 2 and 3), the bulk of the polyp was formed of a peculiar tissue localized to the submucosa. It consisted of fibroblasts and lymphocytes, partly arranged in formations similar to lymph follicles and of numerous, evenly distributed eosinophilic leukocytes. The whole tissue was rich in delicate capillaries and arterioles. Occasionally, cavities with endothelial lining were found, containing a pink substance; they obviously corresponded to lymphatic vessels. The mucous membrane covering the polypus was ulcerated and for the most part replaced by ordinary granulation tissue. The muscularis mucosae was preserved in the stalk only, from which its bundles radiated into the polypoid mass.

### *Case 3*

S. K. was a male, 55 years old, who had contracted a syphilitic infection at age 30 and was treated with neosalvarsan. At about 35 years of age he noticed an indefinite pressure in the epigastrium, especially when his stomach was empty in the afternoons.

He had a feeling of fullness and suffered from belching and sour regurgitation. He often vomited recently taken or partially digested food and bitter-sour fluid. Gastric ulcer was diagnosed, and the complaints disappeared after treatment. Four years before the symptoms had recurred. An operation was suggested but not carried out. A year later he was admitted to the medical clinic owing to a lung complaint. Inflammatory infiltration of the left lung was diagnosed. Roentgenograms of the stomach, taken at the same time, gave the following result: "The stomach showed normal tonus; in the posterior wall of the antrum there was an immovable polypoid formation, the size of a cherry stone. The pyloric passage was free. No retention could be demonstrated, and pressure at the pyloric bulb produced no pain. Three and a half hours after the barium meal, a small remnant was still seen in the stomach while the bulk of the barium had passed into the small intestine." During the patient's further stay at the hospital he did not complain of stomach trouble. On December 10, 1945, the hemoglobin was 105 per cent; erythrocytes, 4,970,000; color index, 1.06; leukocytes, 7,800; differential count: segmented, 55.2 per cent; transitionals, 2.4 per cent; eosinophils, 1.6 per cent; basophils, 0.8 per cent; mononuclears, 8 per cent; lymphocytes, 31.2 per cent; plasmacytes, 0.8 per cent; thrombocytes, normal in number; reticulocytes, 8 per 1,000. Gastric juice (free/total acidity): 32/50, 9/17, 4/13, 30/46, 48/60. The Wassermann reaction was repeatedly positive. Tests for occult blood were positive on several occasions. Blood sedimentation rates were 83, 101, 93, and 103 mm. per hour (Westergren). During treatment with potassium iodide the infiltration of the lung slowly receded and eventually almost disappeared. The patient was then admitted to the second surgical clinic. At that time, the state of nutrition was poor. Percussion of the lungs was normal but there were some ronchi and râles over the left apex of the lung.

In view of the roentgenologic findings, the medical consultants advised operation. A laparotomy was performed under local anesthesia. The stomach was of normal size but there were many thick, flat adhesions, especially on the posterior wall. The duodenum, particularly its bulb, was surrounded and slightly strangulated by firm adhesions which extended to the lower surface of the liver and to the gallbladder. The wall of the latter was not particularly thickened, and no calculi were seen. After separating the adhesions a rigid, localized thickening of the serosa was seen on the anterior wall near the lesser curvature and just above the deformed pylorus. Resection was performed according to Péan-Rydygier. In the submucosa of the prepyloric part a polypoid formation was found.

The postoperative course was uneventful. The patient left the hospital 17 days later. Blood count, taken at that time, revealed: Hemoglobin, 70 per cent; erythrocytes, 3,290,000; leukocytes, 7,600; color index, 1.06; differential count: segmented, 56 per cent; transitionals, 3 per cent; mononuclears, 2 per cent; eosinophils, 1 per cent; basophils, 1 per cent; lymphocytes, 37 per cent.

The patient was seen again 4 months later. He had no complaints, except for persistent coughing. Roentgenograms still showed a shadow over the left apex. Blood counts: Hemoglobin, 65 per cent; erythro-

cytes, 3,200,000; color index, 1.01; leukocytes, 11,800; differential count: segmented, 80 per cent; transitionals, 5 per cent; mononuclears, 0 per cent; basophils, 0 per cent; eosinophils, 0 per cent; lymphocytes, 15 per cent. In the sputum no eosinophilic leukocytes nor Charcot-Leyden crystals were found.

The resected part of the stomach was fixed in a 10 per cent formol solution and submitted for examination. On gross examination (no. 13118/46) no ulcer or scar was visible. In the antrum, 2 cm. from the edge of the specimen, a small polypoid growth was found. Microscopically, the gastric mucosa was practically normal. The polypoid formation was due to an accumulation of abnormal tissue in the submucosa. It had an edematous appearance and was composed of loosely arranged, delicate collagenous fibrils, and of cells with ill defined contours containing elongated, oval nuclei poor in chromatin. Numerous typical eosinophilic leukocytes were distributed in this tissue, particularly in its superficial layers. Lymphocytes, also rather numerous, occasionally showed an arrangement resembling lymph follicles. Besides arterioles and capillaries there were several cavities lined with endothelium. They contained homogenous material staining pink with eosin. The muscularis mucosae covered by the structures described was separated into bundles (Fig. 6), except the most superficial layer, which had preserved its normal parallel arrangement. The peculiar tissue could be traced between the muscle bundles as far as the bottom layers of the mucosa. Toward the surface of the mucosa the eosinophils decreased in number, and the most superficial layer appeared normal except for scattered eosinophils. The pathologic tissue itself showed no sharp lateral delimitation from the normal submucosa. As the whole specimen could not be examined microscopically for technical reasons, the mucosa was carefully dissected from the muscularis, but no further foci of a similar appearance were found.

#### *Case 4*

A. F., a man, 47 years old, had been in apparent good health until the age of 23, when a left-sided herniotomy was performed. At the age of 45 the same operation was performed on the right side. For 10 years he had been complaining of stomach trouble. Three years previously he reported burning and stabbing pains in the epigastrium, and a gastric ulcer was diagnosed. Seven weeks prior to admission he vomited sour fluid stained with bile. Over a short period he lost 8 kg. of weight. Carcinoma of the antrum was suspected and the patient was admitted to the hospital. The epigastrium was tender to pressure. The gastric fluid had normal acidity. The test for occult blood in the stools was negative. The evening temperature was 37.6° C. without any detectable reason. The Wassermann reaction was negative. Blood counts: Leukocytes, 3,100; erythrocytes, 4,800,000; hemoglobin, 90 per cent; color index, 0.93; differential count: segmented, 53 per cent; transitionals, 5 per cent; eosinophils, 3 per cent; lymphocytes, 39 per cent. Roentgenograms revealed stenosis of the prepyloric part of the stomach, believed to be due to an ulcer in view of the

long history and the normal peristalsis in the stenosed part. Delayed evacuation had not been observed. As cancer could not be excluded, operation was advised.

At operation the pyloric part of the stomach was found to be thickened but there was no sign of a malignant growth. On the lesser curvature typical signs of an ulcer were found. Resection of two-thirds of the stomach according to Billroth II was performed.

On gross examination of the resected part (no. 11/45), a deep ulcer was visible at the lesser curvature about 3 cm. above the pylorus. The stomach wall was considerably thickened and scarring extended as far as the pylorus. Near the edge of the ulcer a grayish white, sharply defined structure the size of a pea was found.

Microscopically, a typical deep ulcer with a calloused base was seen. The edges of the ulcer were partly covered with epithelium. The remaining mucosa was infiltrated by lymphocytes and contained many lymph follicles. The muscularis propria was interrupted by the ulcer, and both its ends were typically raised toward the mucosa. On one side the muscularis propria met the muscular layer of the mucosa, and both were directed toward the scar tissue at the base of the ulcer. On the other side the muscularis mucosae was divided into isolated bundles (Fig. 4). Between these and the muscularis propria a peculiar connective tissue growth was present, thrusting the muscular layers apart and reaching up to the mucosa and to the regenerated epithelium at the edge of the ulcer. From the base of the growth it was separated by a thin layer of connective tissue, rich in collagenous fibrils. It was sharply defined from the muscularis propria.

The structure of this pathologic tissue was almost a replica of that seen in the preceding cases, so that a detailed description appears superfluous. The collagenous fibers, however, were noticeably more numerous, and the cellularity somewhat less.

What makes this case particularly remarkable, however, was the fact that the eosinophilic growth was found in the immediate neighborhood of a peptic ulcer of otherwise trivial appearance. One may question whether the growth or the ulcer was pre-existent. The first possibility cannot be excluded although the ulcer was not situated exactly above the growth. If, however, we accept the view that the latter was secondary to the ulcer, it is necessary to stress the fact that the growth was by no means identical with simple granulation tissue such as is commonly seen at the base of ulcers and may contain a certain number of eosinophils.

### *Case 5*

M. B. was a female, 56 years old, with a history of pneumonia in childhood. At the age of 34 she had icterus which reappeared at the age of 46. For 10 years she had been complaining of pressure in the stomach which usually increased after meals. She had suffered from heartburn and belching, and alternative diarrhea and consti-

pation. About 1 year before these symptoms had improved. For the past 2 years she had felt increasingly short of breath, chiefly at night, and complained of stabbing pain in the chest accompanied by a feeling of anxiety. During the past few years she had lost much weight. On admission, the liver was not palpable. In the middle of the epigastrium a resistance was felt which was only slightly tender to pressure. The patient left the hospital in a somewhat improved condition, but 2½ months later she was readmitted because of recurrence of pain and pressure in the epigastrium. Roentgenograms revealed a polypoid formation of the mucosa in the pyloric part of the stomach. In view of the normal acidity of the gastric juice and the normal blood sedimentation rate, cancer seemed improbable. At operation a movable polyp the size of a cherry, with a thin stalk, was removed from the pyloric part of the anterior wall of the stomach. At the same time the gallbladder containing calculi was removed. Four days after operation the patient developed diarrhea and a sharp rise in temperature (40.5° C.). She died with signs of peritonitis.

On gross examination the resected polyp (no. 896/44) was soft and gray. Microscopically, it was covered with normal mucosa. The bulk of the tumor consisted of a tissue showing the same pattern as in the preceding cases, but for minor differences. The network of collagen fibers was noticeably more dense, and infiltration with lymphocytes and eosinophilic cells was less intense. The muscularis mucosae was dissociated into small bundles at the whole periphery of the polypus by the pathologic tissue, which penetrated into the basal layers of the mucosa.

Autopsy (no. 1505/44) revealed a diffuse purulent peritonitis due to infection with *Staphylococcus pyogenes aureus*. In addition to the artefacts produced by the operation, early osteitis deformans of the skull and slight senile atrophy of the brain were found. No further tumor could be demonstrated in the stomach.

### Case 6

P. A. was a female, 56 years old. For the previous 3 months she had complained of frequent spasms of pain in the gastric region, which radiated into the back and under the left scapula. The patient had lost 14 kg. of weight. Physical examination showed tenderness in the epigastric region, where a painful, movable mass was palpable, extending from the borderline of the enlarged liver to 3 fingerbreadths above the umbilicus. Roentgenograms revealed a hypotonic stomach. A dish-shaped, filling defect, 2.5 by 1 cm. wide, with sharply defined borders, was seen. This was situated in the lesser curvature, immediately above the angulus, and close to the anterior wall. The folds of the mucous membrane in the vicinity of the defect were somewhat roughened. Passage through the pylorus was free. A tumor and hypotonic elongation of the stomach were diagnosed.

At operation a tumor was found in the angular part of the lesser curvature, protruding into the lumen. A Péan-Rydygier resection was performed. After the operation the patient developed vomiting and diarrhea followed by dehydration and circulatory collapse. She died 2 days later.

On gross examination the surgical specimen (no. 12811/45) showed a tumorous mass the size of a small walnut, protruding above the level of the mucosa and sharply defined against the muscularis. It was yel-

lowish and soft. Microscopically, the tumor was composed of collagenous fibers with numerous fibroblasts and fibrocytes. Typical eosinophilic leukocytes were evenly spread over the whole tissue but their number was much less than in the preceding cases. Some lymphocytic infiltration was seen also, sometimes forming rudimentary lymph follicles. Occasionally, isolated plasmacytes were found. There were many capillaries and arterioles surrounded by concentrically arranged collagenous fibers and dilated lymphatics were seen as in the preceding cases. The tissue described was situated in the submucosa. It was separated from the muscularis propria by a thin layer of a rather dense and acellular fibrous tissue, and at the lateral borders the collagenous fibers of the submucosa took a course parallel to the surface of the tumor, thus forming a kind of capsule. Toward the mucosa the delimitation was less definite, the muscularis mucosae being separated into single bundles. The mucosa covering the whole area was much thinned out by superficial ulceration, and rather heavily infiltrated with inflammatory cells.

The remaining gastric mucosa showed severe chronic gastritis with widespread metaplasia of the glands to the intestinal type.

At autopsy (24 hours after death) no signs of peritonitis were disclosed. The remaining parts of the stomach contained no tumor. The mucous membrane of the small intestine was covered with mucus.

### DISCUSSION

In all 6 cases a peculiar lesion was found in the submucosa of the stomach. Histologically, it consisted of the following elements: (1) Basic connective tissue composed of mesenchymal elements, *i.e.*, fibroblasts or fibrocytes, and loosely arranged collagenous fibers. In the first 3 cases this tissue showed much edema. (2) Infiltration with eosinophilic leukocytes and lymphocytes, the latter being occasionally accumulated in rudimentary lymph follicles. (3) Arterioles, blood and lymph capillaries.

Clinically, all of the cases have many features in common. In case 1 the growth caused symptoms of stenosis and had to be removed by resection. In case 2 the pain under the processus xiphoideus, occurring especially after meals, was most probably due to the pull exerted by the stalk of the polyp which was seated 2 cm. above the pylorus. The third patient had been complaining for 20 years of symptoms suggesting a gastric ulcer, but in the resected stomach no ulcer was found. In case 4 a calloused ulcer had narrowed the gastric antrum, and the growth in the submucosa at the border of the ulcer may have been aggravating the stenosis. Case 5 was complicated by the presence of cholelithiasis and chronic cholecystitis, but the patient's complaints may have been due, at least partly, to the tumorous proliferation in the submucosa of



the gastric antrum. The sixth patient complained for 3 months of recurring abdominal pain which was obviously caused by the growth in the stomach. It is therefore evident that the process described is by no means a mere accidental finding, but may cause serious symptoms requiring operation.

The gross appearance of the lesion was similar in all 6 cases, although there were some minor differences. The tumor-like tissue was situated mainly in the submucosa, forcing the mucous membrane upwards either as a flat prominence (cases 1 and 6) or in the form of a pendulous polyp (cases 2, 3, and 5). In case 4, which is exceptional in this respect, it was situated at the border of a calloused ulcer. On section the tissue was grayish yellow and soft, having an edematous appearance (cases 1 to 3), or rather whitish, firm, and elastic. In some cases (case 1) the cut surface was studded with small openings which turned out to be dilated lymph vessels.

The microscopic findings in individual cases, though having the features in common as given above, showed variations, which, however, were largely quantitative. The differences concerned the amount of collagenous fibrils and the proportion of infiltrating cells. As a matter of fact, these 6 cases have not been grouped chronologically, but according to these differences. Thus in the first 2 the tissue in question was very loosely built, the collagenous fibrils being scarce, the nuclei of the fibrocytes for the most part vesicular, and the migratory cells, both eosinophils and lymphocytes, fairly numerous. On the other end of the series are cases 5 and 6 with much greater quantity of fibers, more mature fibrocytes, and a smaller number of migratory cells. The remaining 2 cases take an intermediate position.

To evaluate these differences it is essential to attempt a classification of the process. This may be considered as an inflammatory lesion of chronic granulomatous character, or as a neoplastic process, *viz.*, a fibroma, with a secondary inflammatory reaction. If we accept the first point of view, it is clear that the differences may be explained on the basis of the different ages of the process, the highly cellular lesion poor in collagen being the more recent one. If, however, we regard the pathologic new-formation as a fibroma, the differences in cellularity would correspond to various degrees of maturity of the neoplasm.

Personally, I am inclined to consider the process as a granuloma. However, the fact should be stressed that it is by no means identical with the "eosinophilic granuloma," such as is found in bones, and may also occur in soft tissues (Škorpil). There are two main differences between these two lesions, not to speak of localization. In the eosino-

philic granuloma the basic cells are not fibroblasts as in my cases, but histiocytes or reticulum cells. Furthermore, the eosinophils in the first process are densely accumulated in some places while in others they are lacking, whereas in my cases they are evenly distributed throughout the lesion.

To avoid confusion, I therefore suggest—pending a better denomination—the somewhat clumsy term “gastric submucosal granuloma with eosinophilic infiltration” for the lesion described. However, I am aware that in some cases, in which the infiltration with migratory cells has greatly receded, the differentiation from a true fibroma may be rather difficult. In such a case even the criteria given by Hueck may be of little help. In fact, I venture to say that some of the alleged fibromas of the stomach actually were of inflammatory origin.

It is obvious that this question cannot be settled satisfactorily unless the etiology of the lesion is cleared up. In this respect, however, very little can be inferred from my own cases, except that the outstanding eosinophilic infiltration of the pathologic tissue is suggestive of some allergic phenomenon.

Kaijser, in 1937, reported 3 cases of allergic disease of the alimentary tract. Of these, the third case is of special interest as the lesion found in the stomach was studied microscopically. The patient was 53 years old and since his youth had been known to be allergic to onion; he had vomited after eating the smallest quantity. One of his brothers had suffered from attacks of migraine, and one aunt had bronchial asthma. At 23 years he had contracted a syphilitic infection, but the Wassermann test later became negative. He had since received no antisiphilic treatment. For 12 or 13 years he had been complaining of gastric trouble, especially in the spring. The diagnosis of gastric ulcer was made and he was repeatedly treated by diet. The gastric trouble increased during the last 18 months. He complained often of hunger pains which disappeared after a meal, but no hunger pains during the night were reported. Roentgenograms suggested an ulcer of the lesser curvature, about 3 cm. above the pylorus. At operation the pylorus was found to be largely adherent to surrounding structures and the serosa was thickened and scarred. A resection according to Billroth I was performed. A blood count taken 9 days after the operation revealed 26 per cent eosinophilic leukocytes. The resected part of the stomach showed diffuse thickening of the walls, particularly toward the pylorus, which was caused chiefly by edema of the submucosa. Neither gross nor microscopic examination revealed an ulcer. The submucosa of the pyloric part of the stomach was very edematous. In its superficial layer there was considerable inflammatory

infiltration, consisting of neutrophilic leukocytes, lymphocytes, and very numerous eosinophilic leukocytes. This infiltration was most pronounced at the top of the folds of the mucous membrane and increased toward the pylorus. The muscularis mucosae was split up into single bundles. Between these, there was inflammatory edema and considerable infiltration consisting mainly of eosinophilic leukocytes. In places, the submucosa showed very many dilated lymphatic vessels, some of them filled with eosinophilic leukocytes, lymphocytes, and endothelial cells, which last were swollen and proliferating, so that they obliterated the lumen. There was much less inflammatory infiltration in the deeper layers of the submucosa. The muscularis propria showed very slight inflammatory infiltration composed again of eosinophilic leukocytes which were mainly situated along the vessels.

Through the courtesy of Dr. Kaijser I was able to study one original slide from that case. The splitting up of the muscularis mucosae, as described by the author, was clearly seen, but in addition to this there were fibroblastic nuclei, the number of which definitely exceeded what might be expected in pure edema. It therefore appears that the lesion has some similarity to that found in my cases, although in the submucosa there was infiltration with inflammatory cells only, which is at variance with my own findings.

In view of the allergic factor in Kaijser's case, I have carefully reviewed the clinical histories of my cases, but I could not find anything definite in this respect. Nor did the hemograms show anything particular, the number of eosinophils at most approaching the upper limit of normal values. Pending further observations, I therefore feel that the question of etiology is to be left open.

#### SUMMARY

In 6 patients who had suffered from stomach trouble of various kinds, a peculiar lesion was observed consisting of more or less loose collagenous tissue with fibroblasts, lymphocytes, and eosinophilic polymorphonuclear leukocytes. The pathologic tissue thus composed appeared as a circumscribed focus in the submucosa, spreading toward the mucosa of the stomach. Macroscopically, it caused a bulging of the mucosa, and in some cases even a polypous formation. In one case it was situated at the border of a chronic peptic ulcer.

The composition varied somewhat as to the proportion of the collagenous fibrils to the migratory cells, particularly the eosinophils. This seemed to be due to a different degree of maturation of the pathologic tissue.

The lesion is apparently a granuloma, which, however, is definitely different from the "eosinophilic granuloma" of the bone or soft tissues. To point out this difference the provisory term "gastric submucosal granuloma with eosinophilic infiltration" is suggested. In view of a case described by Kaijser, an allergic etiologic factor has been considered.

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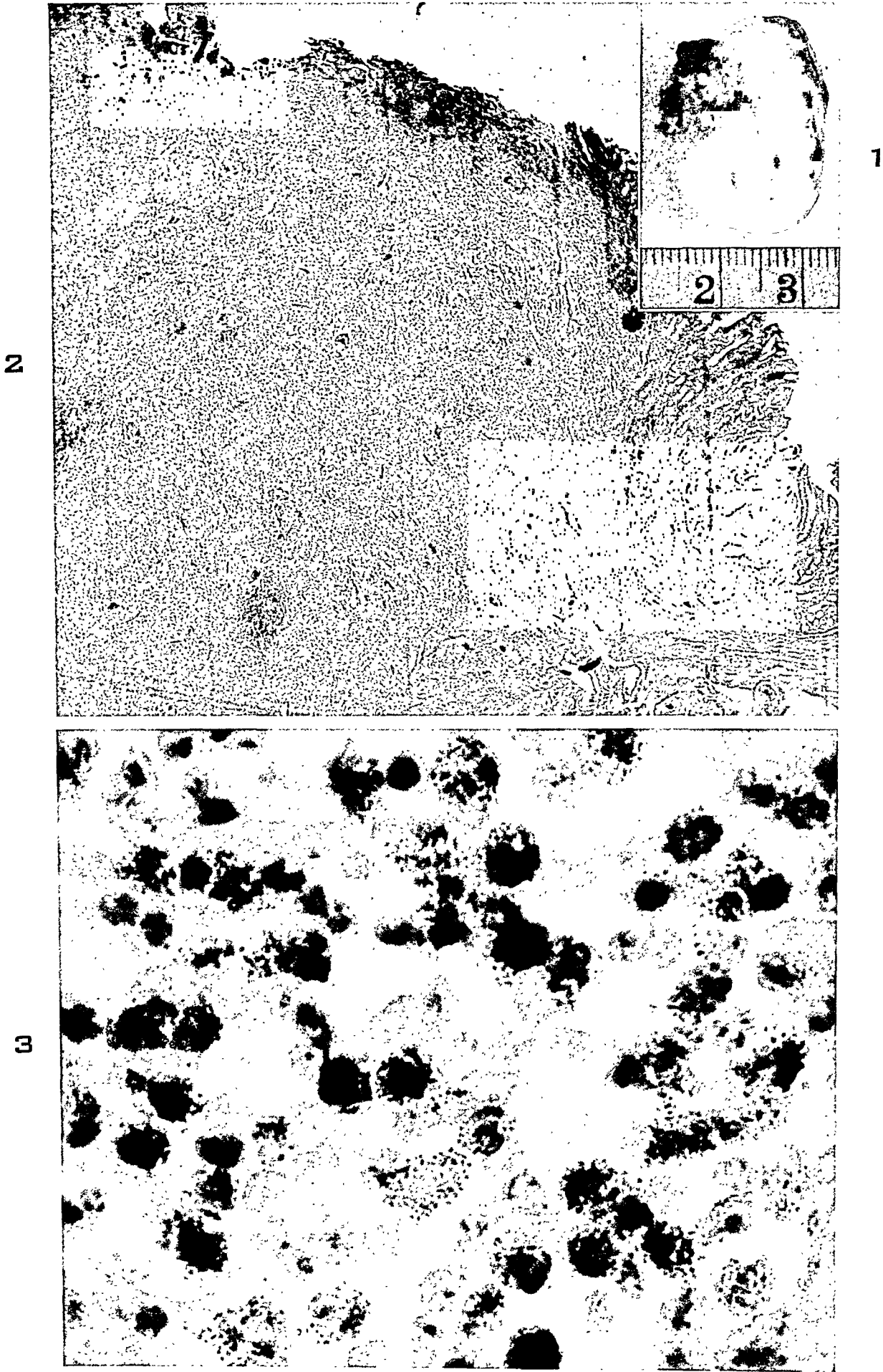
[ Illustrations follow ]

## DESCRIPTION OF PLATES

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### PLATE 56

- FIG. 1. Case 2. Gross specimen of the pendulous polyp found in the antrum.
- FIG. 2. Case 2. Granuloma enlarging the mucosa and infiltrating the mucosa. The dark areas in the granuloma are foci of lymphocytes.  $\times 27$ .
- FIG. 3. Case 2. Higher magnification of the granuloma showing the relatively great number of eosinophilic cells.  $\times 1000$ .



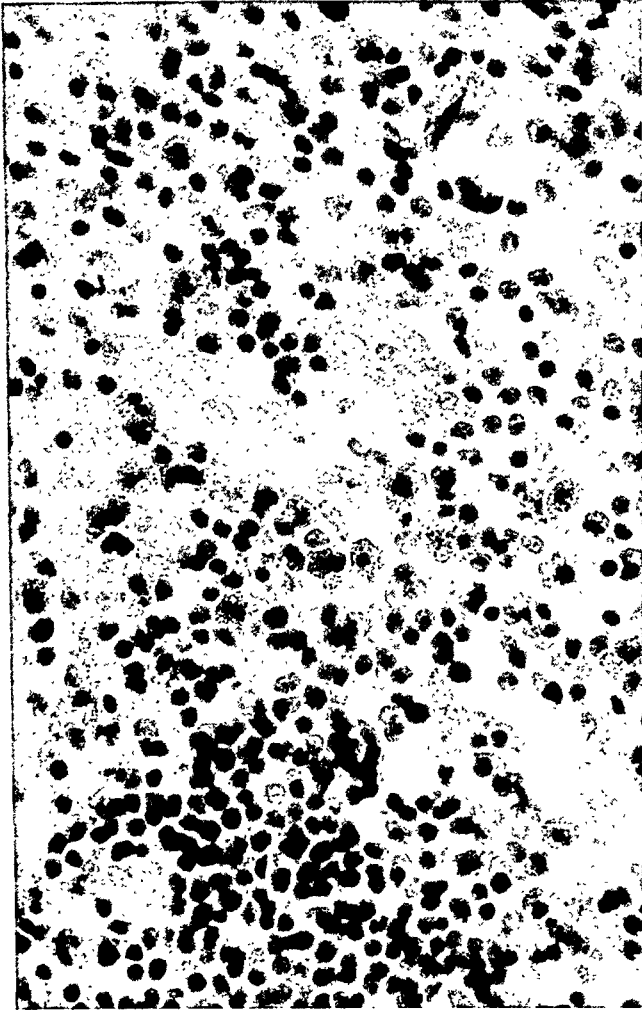
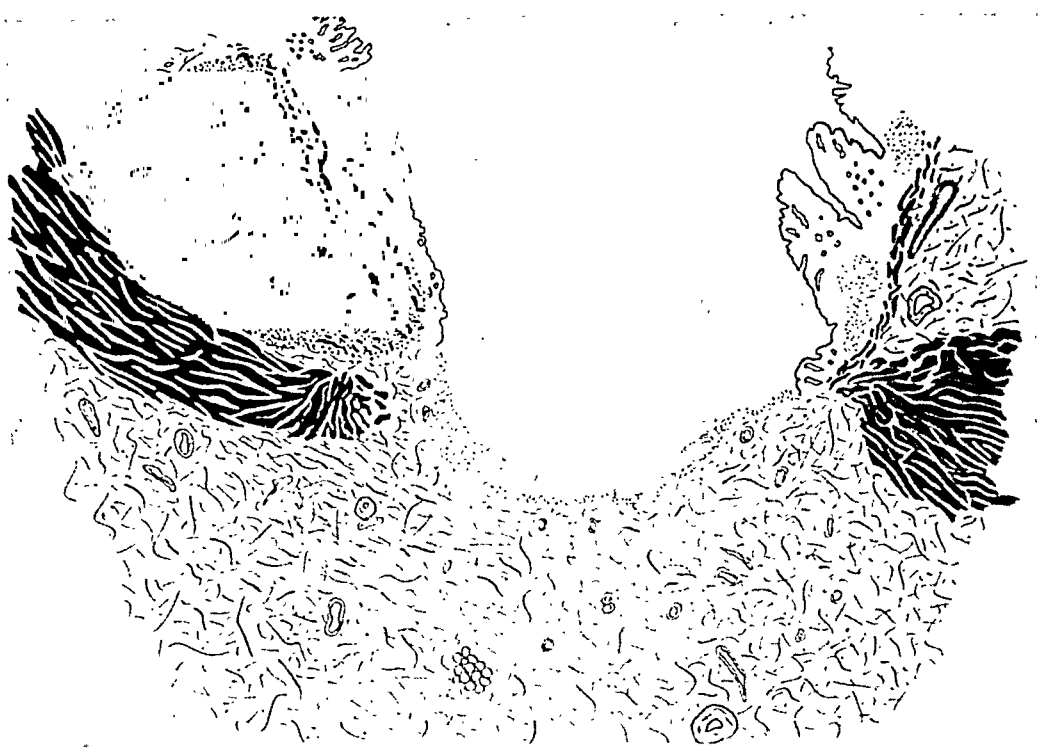
Vaněk

Gastric Granuloma with Eosinophilic Infiltration

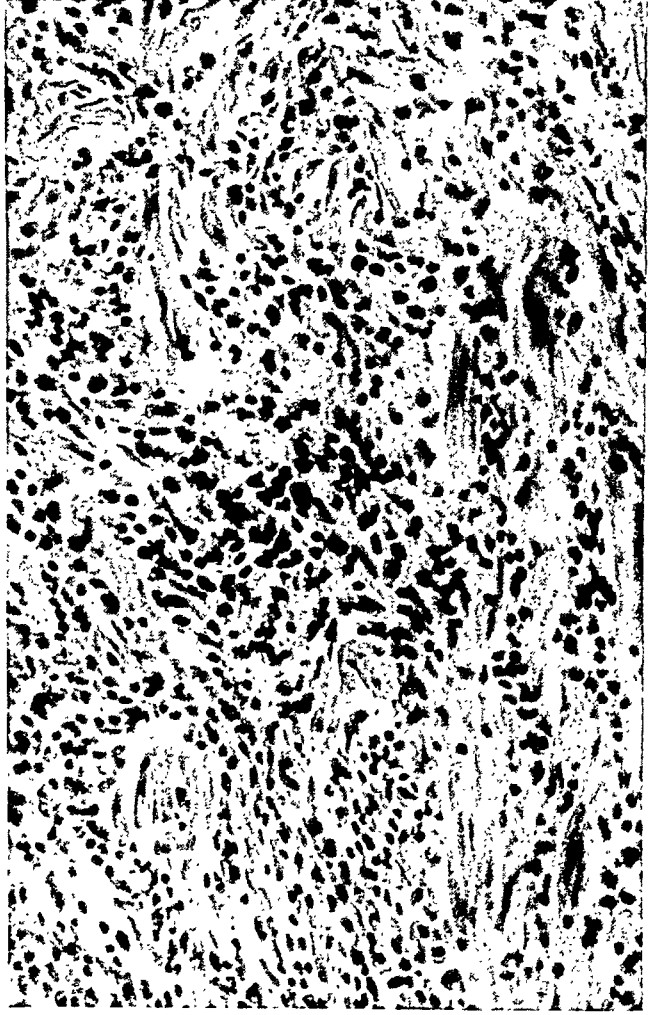
PLATE 57

- FIG. 4. Case 4. Drawing to show the topographic relations of the granulomatous tissue in the submucosa to the peptic ulcer. The muscularis mucosae is split into isolated bundles.
- FIG. 5. Case 1. Typical pattern of the lesion in the submucosa includes fibroblasts with swollen nuclei, eosinophilic leukocytes, and lymphocytes. The lymphocytes are grouped as a rudimentary lymph follicle in the lower portion of the field. Hematoxylin and eosin stain.  $\times 260$ .
- FIG. 6. Case 3. Splitting up of the muscularis mucosae of the stomach into isolated bundles by the granuloma. Heavy infiltration of eosinophils and lymphocytes. Hematoxylin and eosin stain.  $\times 180$ .

4



5



6

Vaněk

Gastric Granuloma with Eosinophilic Infiltration





# LESIONS OF HYPERSENSITIVITY INDUCED IN RABBITS BY MASSIVE INJECTIONS OF HORSE SERUM \*

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It is a familiar thesis that hypersensitivity plays a major rôle in the production of the clinical manifestations and histopathologic changes in a number of diseases.<sup>1,2</sup> Many writers have discussed the importance of hypersensitivity in man in the pathogenesis of rheumatic fever,<sup>3-10</sup> periarteritis nodosa,<sup>11-16</sup> Loeffler's syndrome,<sup>17-19</sup> rheumatoid arthritis,<sup>1,2</sup> and other possibly allied conditions,<sup>19-23</sup> and it has been concluded that certain morphologic changes are characteristic of tissue reactions of hypersensitivity.<sup>19,21,23-25</sup>

This conclusion was based on morphologic studies of lesions attributed to hypersensitivity in man<sup>14,15,19,21,25</sup> and those produced experimentally in animals.<sup>4,5,26</sup> The basic changes which were stressed consisted of evidence of increased capillary permeability, edematous swelling and fibrinoid necrosis of collagen, together with a cellular infiltration of variable quantity and type.<sup>21,24,25,27,28</sup> These collagenous alterations and cellular infiltrations were often of focal character and frequently occurred in relation to small vessels, with or without involvement of the vessel wall itself.<sup>21,29</sup>

Klinge<sup>4</sup> and Vaubel<sup>5</sup> were the first to emphasize the similarities between some of the lesions of rheumatic fever and periarteritis nodosa in man on the one hand, and experimentally induced lesions which they attributed to hypersensitivity to foreign proteins on the other. More recently their work has been repeated and extended. Rich has described periarteritis nodosa in man allegedly due to hypersensitivity to sulfonamides<sup>14,15</sup> or to iodine<sup>16</sup>; and Rich and Gregory<sup>8,9,12</sup> have described, in rabbits treated with massive doses of horse serum, lesions which resembled those of periarteritis nodosa and of rheumatic carditis in man.

The importance of an understanding of the pathology of hypersensitivity is heightened by the growing recognition of a variety of conditions that present the vascular damage and the collagenous necrosis which are often considered to be characteristic of the tissue reaction of hypersensitivity. Because of the basically similar morphologic alterations, some authors<sup>19-21,30</sup> have placed in one group some or all of the following diseases: periarteritis nodosa, rheumatic fever, rheumatoid arthritis, dermatomyositis, scleroderma, and disseminated lupus erythematosus. Teilum<sup>21</sup> and Bergstrand<sup>19</sup> believed that in these conditions there was

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a common pathogenetic relationship based on the reaction of hypersensitive tissues. While hypersensitive tissue response may be regarded as strongly suspect in the rôle of the pathogenetic mechanism in these diseases, the evidence is not as yet conclusive and until it is, they are perhaps better regarded as diseases of unknown etiology and pathogenesis.

Two further reasons exist for exercise of caution in accepting hypersensitivity as the pathogenesis for such lesions on the basis of structure alone. First, there is the failure to find in human cases with well established hypersensitivity lesions which consistently possess the collagenous necrosis common to the preceding group of diseases of unknown etiology; and secondly, there is the wide variation in the structure of the lesions in those cases of established hypersensitivity.<sup>14-16,28-32</sup> In the latter group of cases, sulfonamides, iodine, aspirin, and horse serum have been mentioned as antigenic agents. The wide variety of lesions attributed to one or another of these antigens include: a necrotic and proliferative polyarteritis; granulomata, both paravascular and focal in the heart, liver, and kidney; focal necrosis of the myocardium, liver, and bone marrow; massive necrosis of the liver; and tubular degeneration of the kidney. Of these human lesions with the pathogenesis fairly definitely established to be due to hypersensitivity, only the arteritis shows the collagenous necrosis characteristic of the previously mentioned diseases considered by some to have a common pathogenesis. Furthermore, the only experimental lesion of hypersensitivity which consistently has a similar appearance to possible or proved lesions of hypersensitivity in man is the experimental polyarteritis. However, a recent report<sup>33</sup> of a case of periarteritis nodosa of unknown etiology with granuloma, possibly analogous to the periarterial granuloma of sulfonamide hypersensitivity,<sup>29</sup> may indicate a closer relationship than was previously suspected between the human lesions known to be due to a reaction of hypersensitive tissues and the group of diseases of essentially unknown cause mentioned above.

From the experimental side the precise nature of the lesions which can be attributed to hypersensitivity on a morphologic basis is obscured by a considerable lack of uniformity in the reports of hypersensitive tissue reactions in experimental animals.<sup>4,5,8,14,15,30-32,34-37</sup> The issue becomes still more confused by reports that lesions similar to Aschoff nodules and those of periarteritis nodosa occur in rats following the administration of massive doses of desoxycorticosterone acetate.<sup>38,39</sup> Manifestly, these observations cast some doubt on the actual pathogenesis of lesions hitherto attributed to hypersensitivity, both in man and experimental animals.

As an approach to the solution of some of these problems, we have

carried out a series of experiments somewhat more extensive than previously reported, with a view to determining how great a variety of lesions can be produced in different tissues as a result of hypersensitization induced by massive injections of horse serum. These experiments follow in natural succession those of Loncope,<sup>34-36</sup> Klinge,<sup>4</sup> Vaubel,<sup>5</sup> and Rich and Gregory,<sup>8,9,12</sup> but differ from them in that a standard technic of hypersensitization was employed in a much larger series.

### MATERIALS AND METHODS

The rabbits used in this experiment were derived from several sources. The majority were albinos, although several other strains were included. The proportion of males to females was roughly equal. Their average weight was 1800 to 1900 gm. although a few animals as small as 1400 to 1500 gm. were used.

TABLE I  
*Relation of Incidence of Arteritis to the Number of  
Injections of Horse Serum*

No. of massive injections	No. of animals in group	No. of animals in group with arteritis
1	6	1
2	38	22
3	7	3
4	4	4
6	9	7
7	6	5
8	7	5
Total	77	47

Whole horse serum was used as an antigen. The Department of Microbiology of the University of Montreal very kindly supplied this after separating the serum from the clot. We then passed it through a Seitz filter and stored it in rubber-capped bottles at 4°C. until ready for use. A preservative was not added to the serum, and numerous test cultures taken immediately before use failed to show bacterial contaminants.

Each animal was given an initial intravenous dose of 10 cc. of serum per kg. of body weight. When successive injections were given, they were repeated every 17 to 18 days. Since the animals gained weight, the absolute dose tended to increase, but was arbitrarily limited to a maximum of 20 cc. per injection. To desensitize the animals partially, and to reduce somewhat the mortality rate from anaphylactic shock, the repeat injections were preceded by 24 hours by an intravenous injection of 1 cc. of serum.

The total duration of the experiments varied from 8 to 146 days. Seventy-seven animals received one or more massive injections (Table I).

Eight died immediately of anaphylactic shock, or within a few hours after their last injection. The remaining animals were sacrificed by air embolism, usually 6 to 10 days after the final injection of serum. Complete autopsies were performed and blocks of tissue were fixed in a 10 per cent saline solution of formol. Histologic sections were prepared from brain, spinal cord, larynx, lungs, heart, aorta, liver, pancreas, stomach, intestines, mesentery, kidney, adrenal, testes, ovaries, bone marrow, thymus, spleen, skeletal muscle, and knee joint, and were stained routinely with hematoxylin and eosin. Where indicated, Masson's trichrome stain for connective tissue, Weigert's stain for elastic fibers, Glynn's stain for bacteria, and the phosphotungstic acid stain for fibrin were employed.

Twenty-five animals, derived from the same source as the treated subjects and living under the same conditions, served as controls. They were not treated in any way. They, too, were killed by air embolism and the same tissues examined as in the treated animals.

## RESULTS

Examination of the untreated control animals revealed spontaneous lesions in many organs in a large percentage of animals. Those most frequently encountered were seen in the brain, lung, liver, stomach, intestine, kidney, and testis. The incidence of vascular and focal inflammatory lesions of the brain of the treated and of the control animals was approximately the same as that reported by Bender.<sup>40</sup> Goodpasture<sup>41</sup> has attributed these, as well as a focal nephritis and interstitial pneumonitis, to the organism *Encephalitozoon cuniculi*. The latter lesions were found in about one-fifth of both treated and control animals. Coccidiosis of some degree in the liver of about one-third of both treated and control animals was noted. An unidentified parasite was found in the testis in one instance. Medial sclerosis, sometimes with calcification of the root of the aorta, was noted also in about 30 per cent of the control animals and in 10 per cent of those treated.

The spinal cord, bone marrow, thymus, thyroid, adrenal, pancreatic islet and acinar tissue, and ovaries failed to show any significant lesions in either control or treated animals.

Lesions occurring in the treated animals, but not in the controls, were found in arteries of the heart, lungs, mesentery, stomach, pancreas, liver, and fallopian tubes; in the aorta, and valve rings of the heart, and in skeletal, cardiac and smooth muscle (Table II). Nonspecific inflammatory lesions were found in joints of both treated and control animals, but were more marked in the treated group.

TABLE II  
Incidence of Lesions Found in the 77 Animals Injected with Horse Serum  
but not in Control Animals

Arteries	Coronary arteritis	47	61.1%	Heart	Massive necrosis of myocardium	7	9.1%
	Aortitis	11	14.3%		Lesion in valve ring	10	13.0%
	Pulmonary arteritis	5	6.5%		Lesion in valve cusp*	6	7.8%
	Mesenteric arteritis	4	5.2%	Lungs	Proliferative lesion in vessels	5	6.5%
	Gastric arteritis	3	3.9%		Capillary thrombosis	1	1.3%
	Pancreatic arteritis	4	5.2%	Skeletal muscle	Acute myositis	15	19.5%
	Fallopian tube arteritis	2	2.6%	Smooth muscle	Acute myositis	2	2.6%
	Hepatic arteritis	1	1.3%	Knee joints	Arthritis	6	11.3%†

\* Lesions of this character were found also in the mitral valve of 2 control rabbits.

† Knee joints of only 53 animals were examined.

### Arteries

Of the 77 animals which received one or more injections of serum, 47 showed arterial lesions. Among these, 16 exhibited inflammatory changes which were classified as acute, 34 as subacute, and 15 as healing or chronic. In every animal in which an acute, subacute, or healing arteritis was found, the coronary arteries were involved. In addition, the pulmonary arteries were so involved in 5 cases, the mesenteric in 4, the gastric in 3, the pancreatic in 4, the hepatic in 1, and the arteries of the fallopian tubes in 2 cases. In no instance did the renal or splenic arteries show a similar inflammatory change. Some arteries showed a striking segmental distribution of the damage (Fig. 3).

While an acute arteritis followed the injection of a single massive dose of serum in 16.6 per cent of cases, the incidence of these lesions rose to 58 per cent with 2 injections and to approximately 73 per cent when 3 or more injections were administered (Table I). There was no significant correlation between the total duration of treatment and the histologic appearances of the lesions.

*Acute Arteritis.* The slightest alteration seen was a minimal loss of detail in the media of small arteries without any other disturbances in the arterial wall. This was followed by moderate edema with some distortion of the structure of the three coats of the arterial wall (Fig. 1).

In some of these cases there was added to this minimal disturbance an accumulation of mononuclear cells in the intima and adventitia. A more common and severe degree of damage was indicated by a marked edema of the media which separated the muscle bundles widely and which usually was associated with a marked accumulation of lymphocytes, polymorphonuclear leukocytes, and numerous large mononuclear cells in the intima and media. In addition, there was a marked edema of the subendothelial layer of the intima producing a separation of the swollen endothelial cells from the media (Fig. 2). In some cases the edema was so severe as to reduce the myoplasm to thin strands, leaving swollen vacuolated nuclei as the only prominent remaining structure of the media (Fig. 2). The internal elastic lamina became thin, less refractile than is normal, and lost its wavy contour simultaneously with the above changes (Fig. 2). A further degree of damage to the arterial wall was indicated by the presence of varying amounts of a homogeneous eosinophilic material which gave an altered reaction to some connective tissue stains, and stained selectively with those for fibrin. This material was found principally in the media, to some extent in the intima, and rarely in the adventitia. The least alteration of this character consisted of small blobs of this material in the media, and also within the edematous intima. At this stage it was difficult to decide whether, in the media, this material lay between muscle cells, merely filling the edematous spaces described above, or whether it involved the actual cytoplasm of the muscle cells. When a large quantity was present in the media, it obliterated all structures except the prominent swollen muscle nuclei, and in many cases it formed a large homogeneous eosinophilic lake which appeared to contain only the floating remnants of pyknotic nuclei (Fig. 2). A small patch of this material occasionally was present in the adventitia. In these areas a gradual change in the character of a collagenous fiber was seen as it was followed from a normal into a damaged area. This consisted first of a slight swelling and then of a change to deep eosinophilic homogeneous-staining material. The latter alteration may have been due to an alteration of the substance of the fiber or may have resulted from the covering or infiltration of the fiber with material which stained in the above manner. While alterations occurred in the internal elastic lamina as described above, it remained comparatively intact when other structures had been lost in an extensive fibrinoid necrosis. In the midst of such a change the outline of the internal elastic lamina, considerably thinned and straightened, could usually be seen (Fig. 2). However, in some very extensively damaged areas it was broken and there was a fusion of the homogeneous eosinophilic contents of the media and intima

across this break (Fig. 2). The various changes described above often were found in different segments and coats of the same artery and in different arteries of the same animal (Fig. 3).

*Subacute Arteritis.* The subacute inflammatory reactions in vessels were characterized particularly by proliferative changes of the intimal and adventitial cells, together with a variable amount of cellular exudate consisting mostly of mononuclear leukocytes. Occasionally the leukocytic collections were of paravascular distribution and presented a somewhat granulomatous appearance, which in the heart vaguely resembled Aschoff nodules.

*Chronic and Healing Arteritis.* In the stage of chronic and healing arteritis the intimal layer was irregularly thickened with cellular connective tissue, the cells of the intimal endothelium were somewhat swollen and hyperchromatic, and the media was slightly distorted because of fibrosis. The adventitia was rendered irregularly thick and dense by the presence of collagenous scar tissue, and in this coat lymphocytes and large mononuclear cells were seen. The comparative resistance of the internal elastic lamina to the destructive forces which damage other tissues of the vessel wall was apparent in the healing stage, in which the outline of the internal elastic lamina could be made out in the midst of fibrous proliferative repair in the adjacent media and intima (Fig. 4). With the loss of edema fluid and cellular exudate, the media in some cases assumed an almost normal appearance (Fig. 5). None of the animals was allowed to live long enough after the last injection to determine to what extent healing of these lesions would occur.

Comparable arterial changes were not seen in any of the 25 control animals.

### *Aorta*

Only the first portion of the aortic arch was examined. In 11 of the treated animals an inflammatory reaction of the intima consisting of edema, cellular infiltration, and swelling of the intimal endothelium was observed. The change was diffuse, involving wide areas of the aortic lining and occasionally extending into the coronary ostia to become continuous with an acute necrotizing arteritis of the coronary arteries. The intimal tissues frequently were edematous and sparsely sprinkled with a mixed mononuclear exudate including lymphocytes, plasma cells, and large mononuclear cells (Fig. 6). In only one case did the media appear to be involved. In that instance, small masses of fibrinoid material, pyknotic nuclei, and nuclear fragments, together with occasional polymorphonuclear leukocytes, were observed in a few areas of the inner one-third. These lesions bore no resemblance to the familiar spontaneous



calcification of the media of the aorta of rabbits which sometimes was encountered among the animals of the experimental group.

These changes of the intima and media were not seen in the aortas of any of the control animals, although they too showed calcification of the media in about the same incidence as the experimental group.

### *Heart*

Inflammatory and degenerative lesions of the myocardium and valve cusps were found in both treated and control animals, and of valve rings and coronary arteries in treated animals only.

The inflammatory reactions involving the coronary arteries have been discussed under the heading of "arteries."

*Myocardium.* In the myocardium, diffuse and focal collections of lymphocytes together with scarring and proliferation of connective tissue elements were seen in some degree in virtually all of the animals of both treated and control groups. These changes were comparable to the spontaneous myocardial lesions of rabbits so well illustrated by Miller.<sup>42</sup> In the hearts of 7 treated animals, however, there was observed a massive necrosis of large areas of muscle for which there was no counterpart in the control material. The involved muscle fibers exhibited an acute degenerative change characterized by pyknosis and loss of nuclei, and swelling and fragmentation of the cell body. In addition, these necrotic muscle fibers showed a very pronounced tendency to take up calcium salts. With hematoxylin and eosin stains, the cell fragments, and sometimes whole fibers, were converted into dense, opaque, deep bluish masses as a result of the calcium deposition. The inflammatory reaction in this tissue varied within rather wide limits. Proliferation of connective tissue elements and a moderate infiltrate of mononuclear cells were the usual findings (Fig. 16). Polymorphonuclear leukocytes were extremely rare.

*Valve Cusps.* Localized and somewhat granulomatous appearing lesions were found in the valve cusps of 2 control animals, and in 6 of the treated group. The changes ranged from a localized swelling and edematous separation of collagen fibers in association with a dense infiltrate of lymphocytes and mononuclear cells (Fig. 9), to lesions of a more diffuse character, in which there was considerable thickening of the entire valve cusp by fibroblastic activity and collagen deposition (Fig. 7). In our material, the histopathologic change which most resembled an Aschoff nodule was found in a control animal (Fig. 8), and even here the resemblance was but superficial. There was nothing in the histologic appearance which would serve to distinguish the lesions

in the valve cusps of the treated animals from those of the control series. These changes were found for the most part in the mitral and occasionally in the aortic valve.

*Valve Rings.* In contrast to the myocardium and the valve cusps, lesions were seen in the valve rings of the treated animals only. These lesions, which were found in 10 animals, consisted of subacute and chronic proliferative focal inflammatory reactions. In these areas the collagenous fibers showed swelling and hyaline changes and the fibrocytes showed proliferative activity. In addition to moderate numbers of mononuclear leukocytes and lymphocytes, occasional large cells with 2 to 5 nuclei and an abundant, granular, basophilic cytoplasm were seen in these foci (Fig. 10). Clustering of nuclei in a palisade fashion about a focus of swollen hyaline collagen was seen occasionally (Fig. 11). Comparable areas of focal inflammatory change were not seen in the valve rings of any of the control animals. All of these ring lesions were found in the mitral area except one which was present in the aortic ring.

### *Knee Joints*

Knee joints were not routinely examined during the first portion of the experiment. As a consequence the knee joints from only 14 control and 53 treated animals are reported upon here.

Difficulty in assessing the effectiveness of the treatment in causing inflammatory lesions in the joints was experienced because of the presence of tissue alterations in both control and treated animals. When present, the changes were found most frequently in folds of synovial membrane at the base of the semilunar cartilage and in the angle formed by the fusion of the joint capsule with periosteum. The maximum changes seen in the knee joint of any control animal are represented in Figure 12. The changes seen in the control animals consisted primarily in slight proliferative tendencies in the lining cells of the synovium, together with a slight increase in density and cellularity of the underlying connective tissue. An inflammatory leukocytic exudate was never encountered in the control animals, and constituted one of the chief points which served to distinguish the degenerative changes of untreated animals from the definite inflammatory response seen in the joints of 6 of the treated animals (Figs. 13, 14, and 15).

These 6 animals were the only ones of the treated group showing joint lesions which we attributed to hypersensitivity. Two of these presented deformity and limitation of movement during life, and at autopsy pink, pitted erosions of the articular cartilage were seen. All presented cellular exudates and some hypertrophic villus formation. The duration of

treatment or number of injections was not obviously related to the frequency or severity of the lesions. The tissue lesions were more marked than the most extensive changes of the control group. The lining endothelial cells were swollen and blended indistinguishably with underlying mononuclear cells two to five layers in depth in several cases. The underlying collagen fibers showed swelling and hyalinization, and the increased vascularity was more severe than any seen in the control series. Degeneration of collagen going on to necrosis of the tissue of a villus is shown in Figure 15. The inflammatory exudate consisted of lymphocytes and large and small mononuclear cells, which showed no tendency to cluster into granulomatous nodules. Proliferative activity of fibroblasts was conspicuous. In the most severe lesion taken from one of the animals which showed gross alterations, of which Figure 14 is representative, the general reaction was of a more acute, diffuse inflammatory nature. The subsynovial tissues were edematous, hyperemic, and diffusely infiltrated with inflammatory cells. The swelling resulted in definite villus formation. A few fragmented pyknotic nuclei were seen, and some polymorphonuclear leukocytes. The small muscular arteries were prominent and swollen, although no degenerative or inflammatory changes were seen in their walls.

### *Muscles*

Samples of striated skeletal muscle from the paravertebral region, the laryngeal region, and thigh were sectioned. In the treated group 15 animals showed varying degrees of necrosis. In 10 of these, small clusters or isolated fibers were altered; in 4, numerous small clusters of affected fibers could be found in a single low-power field (Fig. 17); in one, the foci of muscle necrosis became confluent, and large areas of muscle showed acute degeneration and inflammatory changes (Figs. 18 and 19). These muscle changes were characterized by swelling of the fiber with loss of striation and cellular detail, vacuolation, and fragmentation. Perhaps the most striking feature of the histopathologic changes was the opaque bluish red appearance of altered fibers resulting from their pronounced tendency to take up calcium salts. The inflammatory reaction associated with these degenerative changes varied within rather wide limits. In some cases an infiltrate of polymorphonuclear leukocytes brought about a rapid solution of the affected fiber (Figs. 18 and 19). In the neighborhood of those fibers rendered particularly dense by calcific deposits, the inflammatory reaction was often somewhat granulomatous, with proliferation of sarcolemmal nuclei and an abundance of large, occasionally multinucleated, giant cells (Fig. 19).

In the wall of the stomach in 2 cases, and in the smooth muscle of the

small intestine in one case, foci of acute necrosis of muscle fibers were observed. The inflammatory exudate was not as pronounced as that seen in the striated muscle, nor was there any foreign body reaction with the formation of multinucleated giant cells. Allowing for the differences in tissue, it was believed that the focal necrosis of skeletal muscle, cardiac muscle, and the foci of necrosis in the wall of the gastro-intestinal tract were entirely comparable lesions.

In the control group, lesions similar to the above were not found.

### *Lungs*

The pulmonary arteries of 5 animals in the experimental series showed an acute and subacute arteritis which was identical with that seen in the vessels of other viscera (Figs. 2 and 3). In addition, the main branches of the pulmonary arteries in 3 of the above cases showed inflammatory changes of the intimal endothelium and subendothelial tissue similar to those seen in the aorta (Fig. 6).

As well as the above changes, there was observed in 5 instances a lesion which involved vessels, but took the form of a dense accumulation of round and spindle-shaped mononuclear cells in the adventitial and subendothelial layers of the vessel wall (Figs. 20 and 21). This accumulation involved small and medium-sized arteries and veins. While small numbers of lymphocytes were included in the dense mantles of cells about these vessels, the reaction appeared proliferative rather than exudative. The layer of cells present on the inner lining of the vessel wall was occasionally of sufficient thickness to occlude the vessel lumen completely. Fibrinoid necrosis of the vessel wall was not seen in these lesions.

One rabbit received 6 massive injections of serum and died of anaphylactic shock immediately following the injection of the seventh large dose. Most of the pulmonary capillaries in this case contained a dense, somewhat refractile, eosinophilic, homogeneous substance not unlike hyaline thrombotic material. Hemorrhage into the alveolar spaces was not seen, nor were there any leukocytic collections in relation to the capillaries which appeared to be occluded. Seven other animals died in anaphylactic shock, but this change was not identified in these or any other of the experimental animals.

Lesions comparable to the above were not seen in the lungs of any of the control animals.

### DISCUSSION

From the observations of the preceding experiments, it was concluded that we had produced by one or more massive intravenous doses of horse

serum in rabbits an arteritis basically similar to the lesions of periarteritis nodosa in man. Skin tests for sensitivity to horse serum yielded an Arthus reaction in all treated animals, varying from a hyperemic edematous area 1 cm. in diameter to a sloughing necrotic lesion 6 cm. in diameter. No positive result was found in the control areas of the treated animals or in the untreated control animals. This evidence of tissue hypersensitivity indicates that the arteritis may have its pathogenesis in the reaction of hypersensitive tissues. We have observed also in the hearts and in the knee joints of these rabbits, lesions of a rather non-specific inflammatory character but possessing some features compatible with the lesions of rheumatic carditis and rheumatoid arthritis, respectively, and which are similar to lesions which other investigators<sup>4,5,8,9,43</sup> claim to have produced by experimentally induced hypersensitivity to foreign protein. In addition, lesions were noted in the lungs and in smooth, cardiac, and skeletal muscle which have no precise histopathologic counterpart in man. As a group, the experimental lesions encountered in this study were more similar to the lesion-complex of human serum sickness<sup>30-44</sup> than they were to the lesions of any definite spontaneous disease entity of man.

The occurrence of an arteritis in 61 per cent of the 77 treated rabbits in our series confirms the findings of Klinge,<sup>4</sup> Vaubel,<sup>5</sup> Knepper and Waaler,<sup>13</sup> Rich and Gregory,<sup>12</sup> Hopps and Wissler,<sup>43</sup> Fox and Jones,<sup>26</sup> and Apitz,<sup>45</sup> who have described similar lesions as occurring in 30 to 85 per cent of rabbits injected with numerous foreign proteins by a number of parenteral routes. The numbers of animals used in some of these experiments<sup>12,43</sup> were not sufficient to yield a result of statistical significance, and the findings in control animals were not recorded in others.<sup>43</sup> The failure of Smith and Zeek<sup>46</sup> to confirm the observation of Rich and Gregory is probably not significant inasmuch as they utilized the variation of Rich and Gregory's procedure which would be least effective in producing an arteritis in a very small series of animals. A more significant negative result has been reported recently by Alston, Cheng, and Short,<sup>47</sup> who faithfully followed the procedure of Rich and Gregory on a large series of rabbits. This indicates the importance of factors as yet undefined in the development of these lesions. In our experiments, the series was large enough and the incidence of arterial lesions great enough to establish statistically significant results indicating a causal relationship between this method of treatment and the resultant arteritis. While a hypersensitivity response would seem to be the most likely mechanism of this relationship, it may be possible, as Ehrlich<sup>48</sup> has recently re-emphasized, that tremendous doses of antigen as used in

our experiments may actually produce reactions in vessels as a result of a direct injury resulting in breakdown in the defenses rather than by an actual immune response.

The basic morphologic similarity between the experimentally induced arteritis and that seen in periarteritis nodosa was striking, and has already been emphasized by others.<sup>5,12,13,43</sup>

Slight differences between the experimental and human lesions were present. The cellular exudate was composed predominantly of large mononuclear cells. Further, in the rabbits under the conditions of this experiment, the majority of inflamed arteries were found in the heart, while the kidney and spleen were free of such involvement. In man, the distribution of the arterial lesions in the various organs is substantially different.<sup>49</sup> These appear to be minor quantitative rather than qualitative differences, and possibly may be explained on the basis of species differences. Thus, the similarities are sufficient to suggest that the human disease, periarteritis nodosa, may have its pathogenesis in a hypersensitivity reaction.

This is further supported by the frequent association in man of polyarteritis with other definite allergic conditions<sup>1,12,19</sup> and by the observation of the lesions of periarteritis nodosa in cases of drug hypersensitivity<sup>14-16,28</sup> and serum sickness.<sup>31</sup> These facts do not define the cause of periarteritis nodosa, but they do present good evidence that the pathogenetic relationship of hypersensitivity to periarteritis nodosa may involve more than one antigenic etiologic agent. This is in accord with the observations of Knepper and Waaler<sup>13</sup> that a variety of antigens may be utilized to induce hypersensitivity, and result in lesions that are histologically identical. For the present, however, it must be admitted that while a survey of the literature relevant to human periarteritis nodosa is productive of highly suggestive evidence, the number of reports in which a definite relationship between the action of specific antigenic agents and the production of such arterial lesions can be proved is still too small to allow of any sweeping generalizations regarding the exact nature of the relationship of hypersensitivity to the development of the usual case of spontaneous periarteritis nodosa.

While we observed cardiac lesions similar to those reported in the experiments of others,<sup>4,5,8,9,43</sup> our conclusions were not in agreement with these authors, who claim to have induced cardiac lesions in rabbits by means of experimentally induced hypersensitivity, which are similar to those of rheumatic fever in humans. A careful analysis of their work casts some doubt on the validity of the conclusions drawn from some of these experiments. Klinge<sup>4</sup> used only 5 rabbits in that portion of his

experiments in which he describes a carditis. The only convincing photograph is that of an Aschoff-like perivascular scar, while the picture of the valvular lesion shows nothing more than mucoid swelling found in many normal stock rabbits. Vaubel,<sup>5</sup> who listed changes in the heart muscle in 33 of 47 treated rabbits, does not present any relevant illustrations, and much of the description suggests changes now known to be found in normal untreated rabbits.<sup>42</sup> The incidence and precise character of cardiac lesions in the series of Hopps and Wissler<sup>43</sup> seem open to question, since the illustrations of their lesions included the obvious spontaneous myocarditis of rabbits described by Miller.<sup>42</sup> On the other hand, the pictures of valvular and Aschoff-like lesions in the myocardium in Rich and Gregory's paper<sup>9</sup> are admittedly convincing of the similarity of these lesions to those of rheumatic carditis. They found such lesions in 19 of 51 treated rabbits, but failed to report on the incidence of similar lesions in control rabbits living in the animal house at the same time under the same conditions. The latter consideration is important in the light of our observations on the presence of such lesions in control animals in an incidence not significantly different from that in the treated group.

Whatever the etiology and pathogenesis of some of the cardiac lesions which these authors have attributed to hypersensitivity, and which we found in both control and experimental animals, there is no question of the fundamental morphologic similarity between them and the lesions of rheumatic carditis in man. In our material, in both treated and control animals, the myocardium showed foci of spontaneous myocarditis in approximately the same incidence as that reported by Miller.<sup>42</sup> In the valve cusps of the treated animals, inflammatory foci of mononuclear leukocytes and, occasionally, well formed granulomata were seen, but similar lesions occurred in the control animals. The inflammatory foci encountered in the valve rings of 10 treated animals bore some resemblance to Aschoff bodies in that there was swelling and necrosis of collagen fibers, palisading of nuclei, and even the formation of giant cells in small numbers. It is true that similar lesions were not found in the valve rings of the 25 control animals. While the valve rings and valve cusps were considered separately in the tabulation of results, it is not reasonable to regard them as dissociated functional and anatomic entities when the pathogenesis of the inflammatory lesions seen there is considered. The total incidence of inflammatory lesions of the valves and valve rings is 2 in the 25 controls (8 per cent) and 16 in the 77 treated animals (20.8 per cent). While this higher incidence in the treated animals is suggestive, the difference is not actually statistically significant. Thus,

while it is apparent that the valvular cardiac lesions that we have described are in some respects similar to those of rheumatic carditis in man, we are unable to conclude that they were lesions of hypersensitivity; but it must be recognized that species differences might well result in considerable morphologic dissimilarities between the lesions in the rabbit and in man, and that a suitable variation of this experimental procedure might considerably reduce the dissimilarity and increase the frequency of such experimental lesions. The fact remains that under the conditions of these experiments our observations have failed to prove that lesions with the characteristics of rheumatic carditis can be produced experimentally in rabbits by this method. Moreover, it appears to us that no other experiments have as yet yielded unmistakable proof that such lesions may be induced on the basis of a reaction of hypersensitive tissues. The similarity of the lesions in control and experimental animals to those of rheumatic carditis, while not giving evidence as to their cause, does suggest that the rabbit is capable of reacting to injury with the production of lesions of this character.

In the lungs the rather unusual changes in vessels, characterized by dense perivascular and intimal collections of large mononuclear cells, were found in 5 of the treated animals, and were not seen in any of the control group. Pentimalli<sup>50</sup> described and illustrated similar lesions in the lungs of rabbits following the parenteral administration of foreign serum. While the percentage incidence of this type of lesion in our material was so small as to raise some reasonable doubt, hypersensitivity cannot be ruled out as the pathogenic agent in their production until the study of larger series is made. Another lesion, found in only one rabbit, consisted of changes involving pulmonary capillaries. In a series of 26 animals, Knepper and Waaler<sup>13</sup> reported the occurrence of hyaline thrombi in the pulmonary capillaries of 8 animals dying of anaphylactic shock. Gregory and Rich<sup>10</sup> studied the lungs of 56 rabbits which had received large intravenous injections of horse serum, and described the presence of focal capillary damage in 10 with capillary thrombi in 9. Of this group, 2 animals died in acute anaphylactic shock, 4 more died within 24 hours, and 3 lived several days after a shocking dose of horse serum. Gregory and Rich believed that these lesions were comparable to those of pneumonitis in acute rheumatic fever and the pulmonary changes of sulfonamide hypersensitivity. In our experiments, 8 animals died of the effects of anaphylactic shock, and of these, one showed capillary plugs of hyaline material not unlike thrombus material. Comparable changes were not seen in any other animals. In some of the lesions illustrated by Gregory and Rich, an early, slight leukocytic



response is seen. Our isolated case showed no such inflammatory reaction. It would seem reasonable that if this histopathologic change is to be regarded as a part of a hypersensitivity reaction, it must be with the proviso that its occurrence seems to be associated with severe anaphylactic reactions, frequently involving the death of the animal.

In addition to the massive necrosis of heart muscle which was observed in 7 animals, and necrosis of striated muscle in 15, necrotizing lesions of smooth muscle were observed in the stomach wall in 2 cases and intestinal muscle in one case. These lesions were found only in the treated animals. The leukocytic reaction in the striated muscle lesions was more pronounced than that seen in the heart and intestine, but we feel that if some allowance is made for tissue differences, the three lesions might be considered to be basically the same. The illustrations of Klinge<sup>4</sup> and of Vaubel,<sup>5</sup> who produced these lesions in small numbers of skeletal muscle fibers close to joints following multiple serum injections, are qualitatively similar, although apparently not as extensive or as severe as those seen in our material. More recently Steiner, Freund, Leichtentritt, and Maun<sup>51</sup> have described focal inflammatory lesions of skeletal muscle and peripheral nerves in cases of rheumatoid arthritis, but Clawson, Noble, and Lufkin<sup>52</sup> found similar lesions in control autopsy material. While hypersensitivity may be a factor in the development of such lesions, their lack of specificity is further indicated by the fact that they have been found in rabbits under such a wide variation of circumstances as cholesterol feeding<sup>53</sup> and infection and sensitivity to streptococci.<sup>54</sup>

Many opinions exist, some supported by experimental observations, regarding the cause of rheumatoid arthritis.<sup>4,55-60</sup> Our experimental findings, like Klinge's,<sup>4</sup> indicate a possible relationship between hypersensitivity and the development of chronic inflammatory arthritis in rabbits. While the lesions described were nonspecific and represented, for the most part, a quantitative change from abnormalities seen in the joints in control animals, it is important to note that a definite inflammatory component was added to the changes seen in the knee joints of 6 of the treated animals, and in one of these degeneration of connective tissue was present similar to the fibrinoid necrosis of arterial walls. Furthermore, 2 of the animals presented clinical and pathologic features similar to those of well advanced active rheumatoid arthritis in man. In these there were pannus formation, pink, pitted erosion of the articular cartilage, limitation of movement, distortion, and disorganization of the joint. Unfortunately, the results do not warrant an absolute conclusion concerning the relationship of the experimentally induced hypersensi-

tivity to the occurrence of the joint lesions. Turnbull's description<sup>60</sup> of the remarkable improvement in many cases of rheumatoid arthritis in human beings when put on diets free of foods to which they were sensitive, indicates strongly the importance of hypersensitivity in the complex etiology of rheumatoid arthritis.

The foregoing discussion brings out rather convincing evidence that an arteritis and possibly lesions of the valve rings and knee joints of rabbits can be produced by massive intravenous injections of horse serum. In view of this it is interesting that Alston *et al.*<sup>47</sup> failed to produce an arteritis in rabbits in an adequate experiment using identical methods of treatment. This indicates the importance of other factors in the development of such an arteritis in rabbits made hypersensitive to horse serum. There remains the question of whether the fibrinoid degeneration of these lesions is a specific manifestation of a reaction of hypersensitive tissues, and the question of the relationship of these experimental lesions to lesions of somewhat similar morphology found in a variety of diseases in man. It is pertinent to pursue these two questions somewhat further.

A marked fibrinoid necrosis of the connective tissue and related structures was a prominent feature of the majority of the well advanced arterial lesions. Whether this latter change was the result of a flooding of the damaged tissues with a material that stained selectively for fibrin or whether the eosinophilic substance represents greatly swollen or much increased degenerated ground substance, collagen, and myoplasm, could not be determined. Whatever the origin of this material, it was associated with rather widespread necrosis of tissues in the areas where it was found. There is no reason to believe that the fibrinoid necrosis of these lesions is in any way specific for the reaction of hypersensitive tissues because, as Klemperer<sup>61,62</sup> has pointed out, similar changes are found in the course of some acute bacterial infections, in necrotizing arteritis of experimental hypertension, inflammatory lesions due to physical and chemical irritation, areas adjacent to pancreatic fat necrosis, the base of peptic ulcers, and in some experimental traumatic lesions of skin. It seems more reasonable to believe, as Duff<sup>63</sup> has pointed out, that this alteration represents the final outcome of changes occurring in connective tissue as a result of a variety of injurious agents and conditions. It would be unwise, therefore, to generalize regarding hypersensitivity as the possible basis for a variety of human diseases because of morphologic similarities between the lesions of these diseases and the experimental lesions of hypersensitivity. For this reason, it seems rather premature to consider the healing arteritis of coronary arteries of Rich and Gregory's sensitized rabbits as related to the "sclerotic" type of

arteritis of rheumatic fever and disseminated lupus erythematosus.<sup>22</sup> Also, from our own observations such an assumption seems unwarranted because we have seen such an arteritis in the mesenteric arteries of our sensitized rabbits (Fig. 5), which is obviously the healing stage of an acute arteritis (Fig. 2), and which, if it can be compared to anything in the human, is to be compared only to the lesion of healed periarteritis nodosa. Furthermore, such caution in interpreting the relation of hypersensitivity to lesions of this character is in line with the facts recently presented by Baehr and Pollack,<sup>64</sup> who stressed the lack of substantial evidence for the hypersensitive nature of disseminated lupus erythematosus and scleroderma.

So far as our material was concerned, it was not clear that the lesions resembling rheumatic carditis morphologically were due to experimentally induced hypersensitivity. Rössle,<sup>23</sup> Friedberg and Gross,<sup>65</sup> and Teilum<sup>21</sup> have analyzed groups of cases of combined periarteritis nodosa and rheumatic fever and have critically discussed this association and the blending of one lesion into the other in the heart in these two diseases. Rich and Gregory<sup>8</sup> have summed up rather convincing evidence to show that in the human, many cases of rheumatic fever present manifestations of anaphylactic hypersensitivity. The clinical association of human periarteritis nodosa and rheumatic fever, and the demonstration that periarteritis nodosa in man and similar experimental lesions in rabbits can result from serum hypersensitivity, constitute rather suggestive evidence for the view that they may well result from a common or closely related pathogenetic mechanism. Therefore, in spite of the fact that it was impossible to conclude from our results that rheumatic-fever-like lesions may be induced in experimental animals by this method, and although the experimental proof of others is not convincing on this point, we believe, nonetheless, that none of the evidence precludes the possibility of a causal relationship between hypersensitivity and rheumatic fever, and it seems reasonable that further investigations by other methods of inducing hypersensitivity may well result in a more clearly defined relationship. What has been said of rheumatic fever can be said equally of rheumatoid arthritis and the relation of our experimental joint lesions to this disease. In this connection, recent support has been given to the view that antigens of differing types and differing methods of administration are associated with immune responses<sup>66,67</sup> of differing types and that the lesions resulting from sensitization vary with variation in the immunization procedure.<sup>68-74</sup>

Even if it can be demonstrated that tissue hypersensitivity plays a determining rôle in rheumatic fever and possibly in rheumatoid arthritis,

we should still bear in mind what has already been emphasized by Aschoff<sup>7</sup> in regard to rheumatic fever, that if such a relationship exists, it can be only a pathogenetic one and the etiology will remain unsolved. Rackemann<sup>55</sup> has discussed the possible diversity of the antigenic component of such a relation and the importance of many complex local and general factors, such as inherent and changing constitution, in determining when reactions will occur, what their structure will be, and in what tissues they will develop. Such modifying factors must be taken into account before we can accept intelligently the hypothesis of hypersensitivity as an explanation for the development of periarteritis nodosa, rheumatic fever, and rheumatoid arthritis.

### SUMMARY AND CONCLUSIONS

A wide variety of lesions have been attributed to experimental hypersensitivity and in some instances similar experimental lesions have been produced by other means. In man many diseases associated with lesions of fibrinoid necrosis of collagen have been attributed to reactions of hypersensitive tissues. In view of the problems posed by these facts we undertook to determine the variety and frequency of lesions that would occur in a large group of animals made hypersensitive by one method. Seventy-seven rabbits were sensitized to horse serum by means of one or more intravenous doses of 10 cc. of fresh sterile horse serum per kg. of body weight. They were sacrificed approximately 10 days after the last injection, and the brain, heart, aorta, lungs, liver, spleen, pancreas, stomach, intestines, mesentery, kidneys, skeletal muscle, knee joint, bone marrow, and thymus were studied histologically.

An arteritis morphologically similar to periarteritis nodosa was found in 60 per cent of the rabbits. While there seemed no reason to doubt a causal relationship between the arteritis and the injections of horse serum, it was pointed out that the failure of Alston *et al.* to confirm these results indicates the importance of other factors in the development of these lesions.

Our observations did not confirm the conclusions of Rich and Gregory that lesions similar to rheumatic carditis can be produced in rabbits by massive intravenous doses of horse serum. It is true that inflammatory lesions of heart valves were seen in 20 per cent of the animals which received injections of horse serum, but similar lesions were found also in 8 per cent of the control rabbits, and the one most resembling a lesion of rheumatic carditis was found in a control animal. Furthermore, no Aschoff-like lesions in the myocardium were seen. Our observations indicate that if lesions in the rabbit like those of rheumatic carditis are

a result of hypersensitivity, some method other than that used in our experiments will be necessary to demonstrate such a relationship.

Of the 53 treated animals whose knee joints were examined, 6 presented inflammatory and degenerative lesions of more severe degree than were found in the control rabbits. However, the incidence was not sufficient to indicate a causal connection between these changes and the administration of horse serum.

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[ Illustrations follow ]

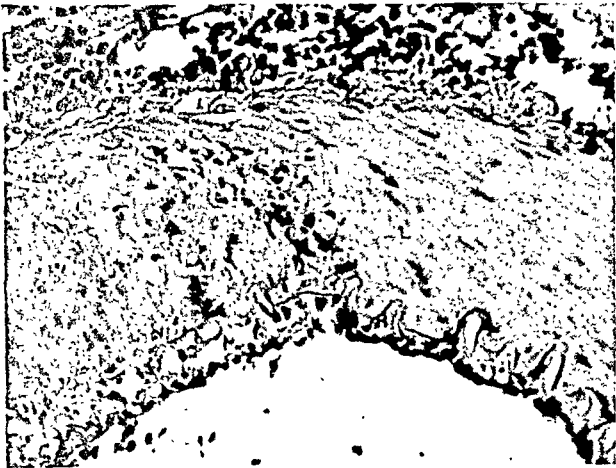


## DESCRIPTION OF PLATES

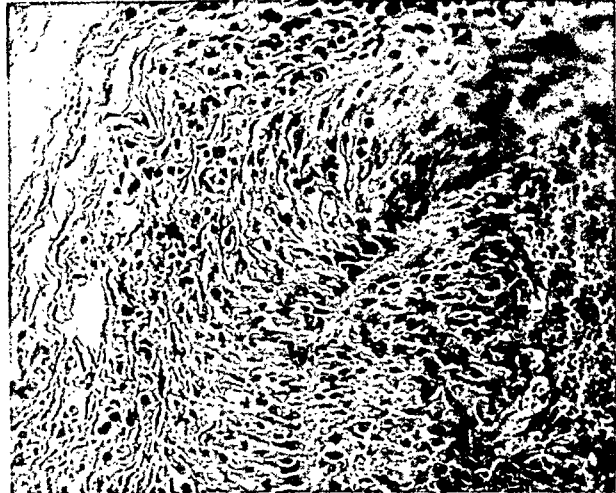
### PLATE 58

- FIG. 1. Acute arteritis of a mesenteric artery of rabbit 48. This animal received 2 injections and was killed 10 days after the second. To the right the media appears normal. To the left the media shows a moderate edema and the presence of some pyknotic nuclei. There is a mononuclear exudate in the adjacent intima and media which extends to the adventitia and intima overlying normal-appearing media. Hematoxylin and eosin stain.  $\times 200$ .
- FIG. 2. Acute fibrinoid necrosis of a mesenteric artery of rabbit 6. This animal received 2 large injections of serum and died of the results of anaphylactic shock several hours after a third injection of 1 cc. The entire wall is involved in an acute degenerative and inflammatory process. The attenuated straightened internal elastic membrane is seen toward the left. The media in the lower part of the photograph shows marked edema. Muscle nuclei and strands of myoplasm are all that can be seen of the media in this area. In the upper field a homogeneous, deeply eosinophilic material which stains selectively for fibrin is present. Remnants of nuclei are seen floating in it. This same material is present in the intima which also shows an accumulation of mononuclear cells. In this region the internal elastic lamina is broken and the eosinophilic material of the media and intima blend. Hematoxylin and eosin stain.  $\times 265$ .
- FIG. 3. Acute arteritis of a medium-sized mesenteric artery of rabbit 48. This animal received 2 injections of serum and was killed 10 days after the second. The segmental character of the reaction can be well seen, with normal appearing areas alternating with damaged segments of the arterial wall. Hematoxylin and eosin stain.  $\times 70$ .
- FIG. 4. Preservation of the internal elastic lamina in healing arteritis of rabbit 114. The lumen containing red blood cells is in the upper left corner. Adjacent to the lumen there is cellular infiltration and proliferation. The definite outlines of the internal elastic lamina, although altered, are seen separating the intima and the media. A part of the media shows edema and fibrous proliferation. Hematoxylin and eosin stain.  $\times 160$ .
- FIG. 5. Chronic arteritis of a medium-sized mesenteric artery of rabbit 27. This animal received 8 injections of serum and was killed 10 days after the last injection. The endothelial cells tend to be swollen and prominent, and the subendothelial layer is markedly thickened with cellular connective tissue. The media shows slight distortion due to scarring. Lymphocytes and large mononuclear cells are plentifully sprinkled throughout the rather dense collagenous adventitia. Hematoxylin and eosin stain.  $\times 68$ .

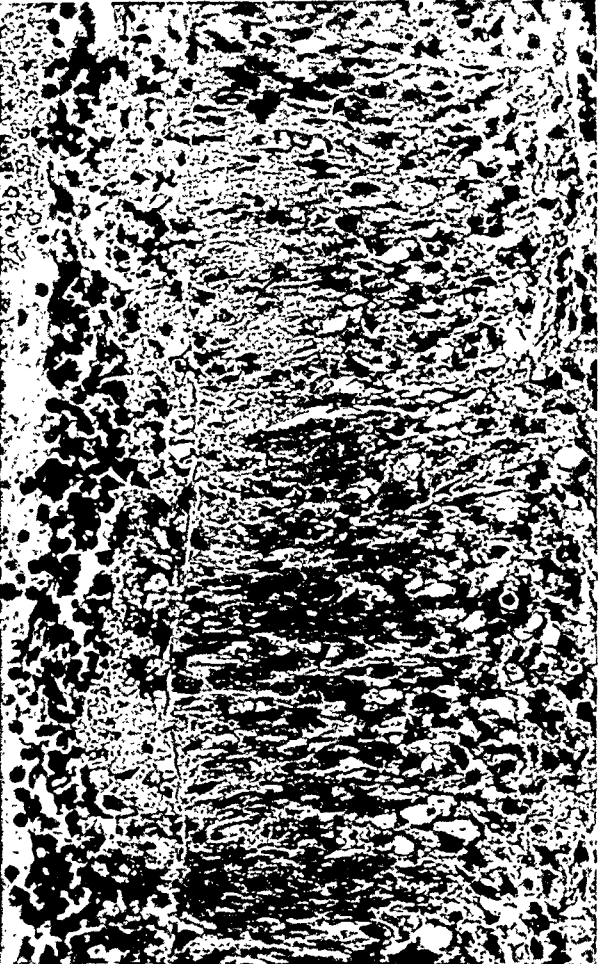
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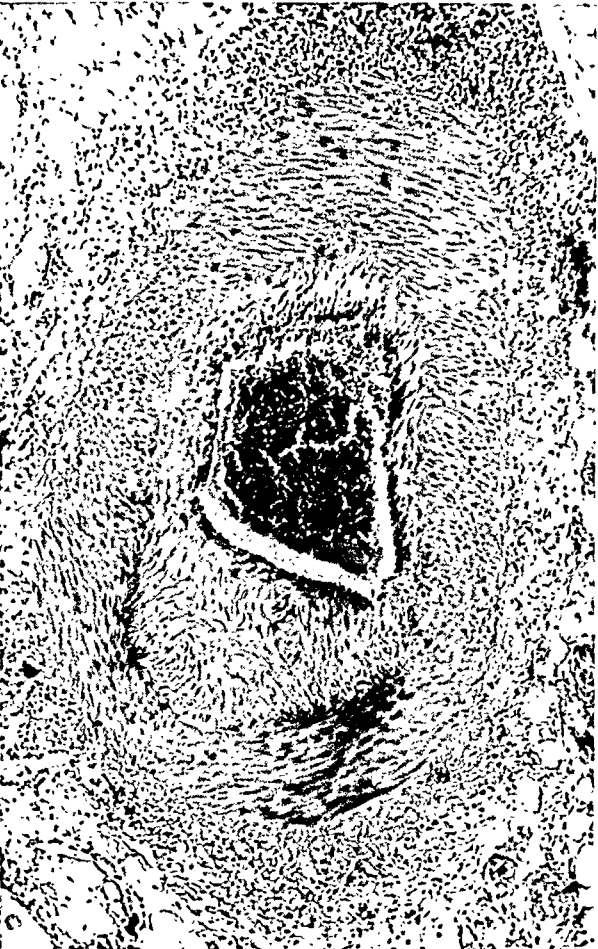


PLATE 59

- FIG. 6. Diffuse inflammatory reaction in the subendothelial tissue of the aorta of rabbit 58. This animal received 2 injections of serum and was killed 4 days after the last injection. A diffuse infiltrate of lymphocytes and mononuclear cells is seen in the subendothelial tissue. Hematoxylin and eosin stain.  $\times 100$ .
- FIG. 7. Diffuse inflammatory reaction in the heart valve of rabbit 27. This rabbit received 8 injections of serum and was killed 10 days after the last injection. The free end of the valve cusp shows a marked degree of diffuse thickening, with excessive deposition of collagen, some fibroblastic activity, and collections of lymphocytes and mononuclear cells. Hematoxylin and eosin stain.  $\times 75$ .
- FIG. 8. Granulomatous nodule in the heart valve of control rabbit 119. An inflammatory nodule is present on the inferior surface of the valve cusp. The central portion of the nodule is pale, with edema fluid which separates the attenuated collagen fibers and contains a few inflammatory cells, chiefly lymphocytes and mononuclear cells. Swelling and proliferative changes are seen in the fibroblasts at the periphery of the lesion. This animal was not treated in any way. Hematoxylin and eosin stain.  $\times 235$ .
- FIG. 9. Focal inflammatory reaction in the heart valve of rabbit 40. This rabbit received 6 injections of serum and was killed 10 days after the last injection. The valve is edematous, pale, and swollen to a fusiform outline. In the central portion toward one surface is a dense collection of small round cells, the majority of which are lymphocytes. Fibrinoid necrosis of the ground substance is not present. Hematoxylin and eosin stain.  $\times 140$ .

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More and McLean

Hypersensitivity Induced by Horse Serum

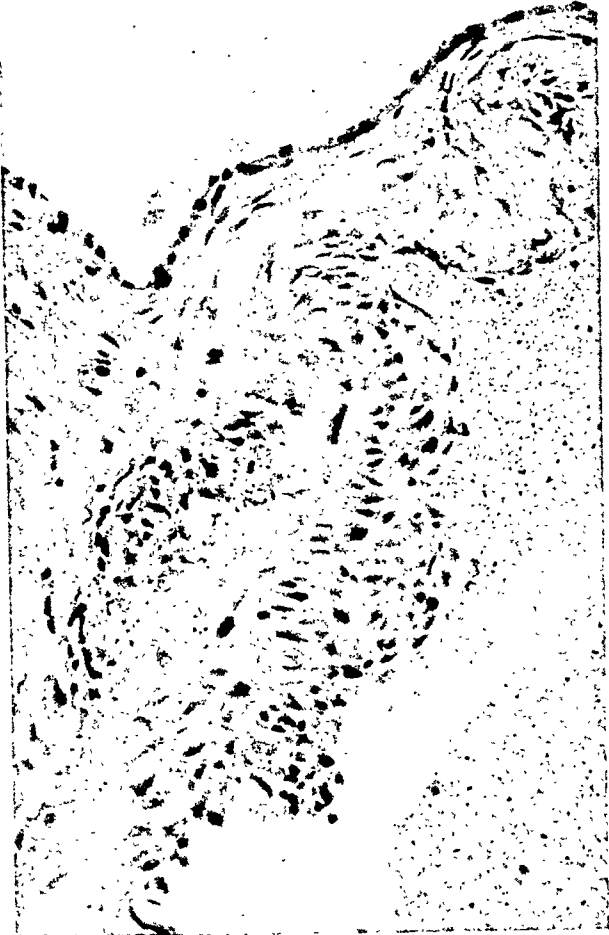
## PLATE 60

- FIG. 10. Granuloma of a mitral valve ring of rabbit 72. There is edema of the connective tissue in the base of the mitral valve with fibrous thickening of the subendothelial tissue. Mononuclear and multinuclear giant cells are prominent. Hematoxylin and eosin stain.  $\times 250$ .
- FIG. 11. Inflammatory nodule in a valve ring of rabbit 15. This animal received 3 large injections of horse serum and died in anaphylactic shock following the third. There is a proliferative inflammatory reaction about a mass of swollen hyaline collagen in the superficial portion of the valve ring beneath the attachment of the valve cusp. The fibroblastic nuclei are swollen and hyperchromatic and show some tendency toward palisading. Hematoxylin and eosin stain.  $\times 250$ .
- FIG. 12. Maximum alteration of the synovial lining of a knee joint of control rabbit 13. There is some subendothelial hyalinization and a slight increase in the cells of this region. Hematoxylin and eosin stain.  $\times 225$ .
- FIG. 13. Moderate change in the synovial lining of a knee joint of treated rabbit 72. This is a villous formation showing edema and a mononuclear infiltration most marked toward the surface. Hematoxylin and eosin stain.  $\times 225$ .

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## PLATE 61

- FIG. 14. Joint capsule of rabbit 27. This rabbit received 8 injections of serum and was killed 10 days after the last injection. There is marked edema of a synovial fold. There is proliferation of the synovial lining cells. The underlying tissue is edematous, hyperemic, and infiltrated with plasma cells, large mononuclear cells, and occasional polymorphonuclear leukocytes. Hematoxylin and eosin stain.  $\times 265$ .
- FIG. 15. Fibrinoid necrosis of a villus from a knee joint of treated rabbit 27. Some connective tissue structure and blood vessels can be made out near the center of the field. About this all structure is lost and the homogeneous material contains fragmented pyknotic nuclei. This homogeneous material was deeply eosinophilic and characteristic of the changes of fibrinoid degeneration. Hematoxylin and eosin stain.  $\times 145$ .
- FIG. 16. Necrosis and calcification of the heart muscle of rabbit 19. This animal received 2 injections of serum and died 7 days after the second. The darkly stained fibers are necrotic, and are stained a deep bluish color with hematoxylin and eosin. This change in staining reaction was due largely to calcium deposits, as demonstrated by the use of von Kossa's stain. Hematoxylin and eosin stain.  $\times 210$ .
- FIG. 17. Necrosis and calcification of the skeletal muscle of rabbit 44. This animal received 2 injections of horse serum and was killed 4 days after the second. Numerous fibers, singly and in small clusters, show varying degrees of degenerative change, with swelling, loss of striations, fragmentation, and intense basophilia due to deposits of calcium salts. Under the microscope, swelling and proliferation of the sarcolemmal nuclei may be seen. Hematoxylin and eosin stain.  $\times 175$ .
- FIG. 18. Necrosis, inflammation, and calcification of the skeletal muscle of rabbit 7. This animal was given 4 injections of serum and was killed 10 days after the last injection. There are many necrotic dark fibers containing calcium, and extensive areas of interstitial infiltration with mononuclear cells and a few polymorphonuclear leukocytes. Hematoxylin and eosin stain.  $\times 180$ .

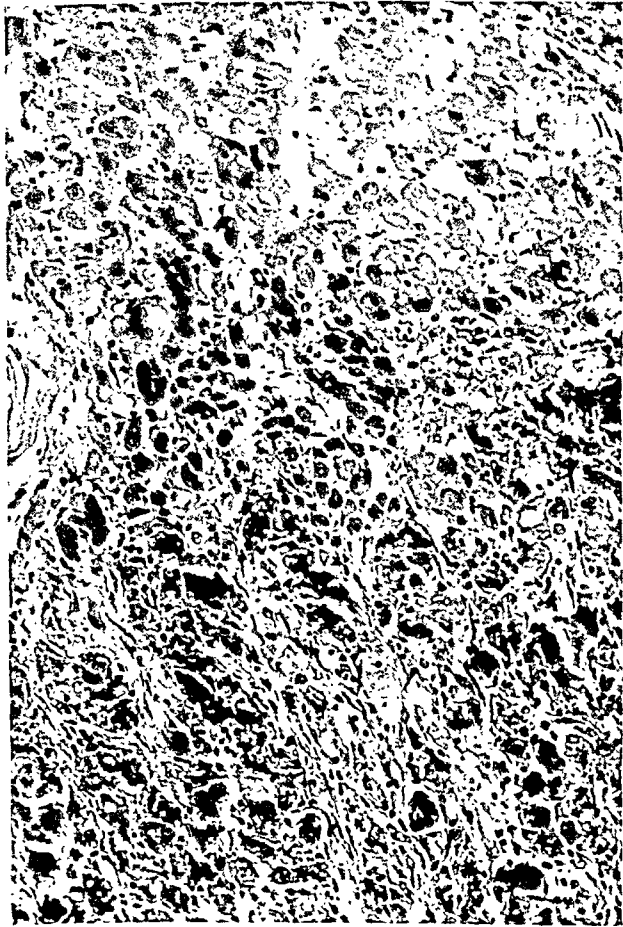
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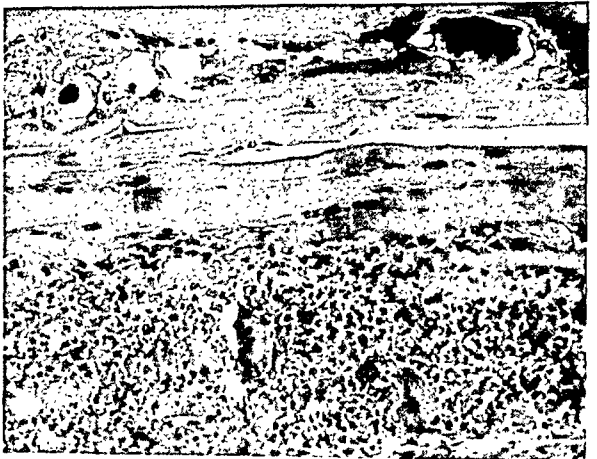




PLATE 62

- FIG. 19. Higher magnification of Figure 18. The character of the cellular proliferation and infiltration can be seen. Hematoxylin and eosin stain.  $\times 620$ .
- FIG. 20. Paravascular granulomas of the lungs of rabbit 55. This animal received 2 injections of serum and was killed 2 days after the second injection. There are dense paravascular collections of cells. In some, the vessel walls can be seen. Hematoxylin and eosin stain.  $\times 49$ .
- FIG. 21. Higher magnification of Figure 18. The thin, irregular vessel wall can be seen and in a few areas appears to be completely destroyed. The character of the large mononuclear cells can be seen. These lie both within and outside the vessel wall. Hematoxylin and eosin stain.  $\times 335$ .

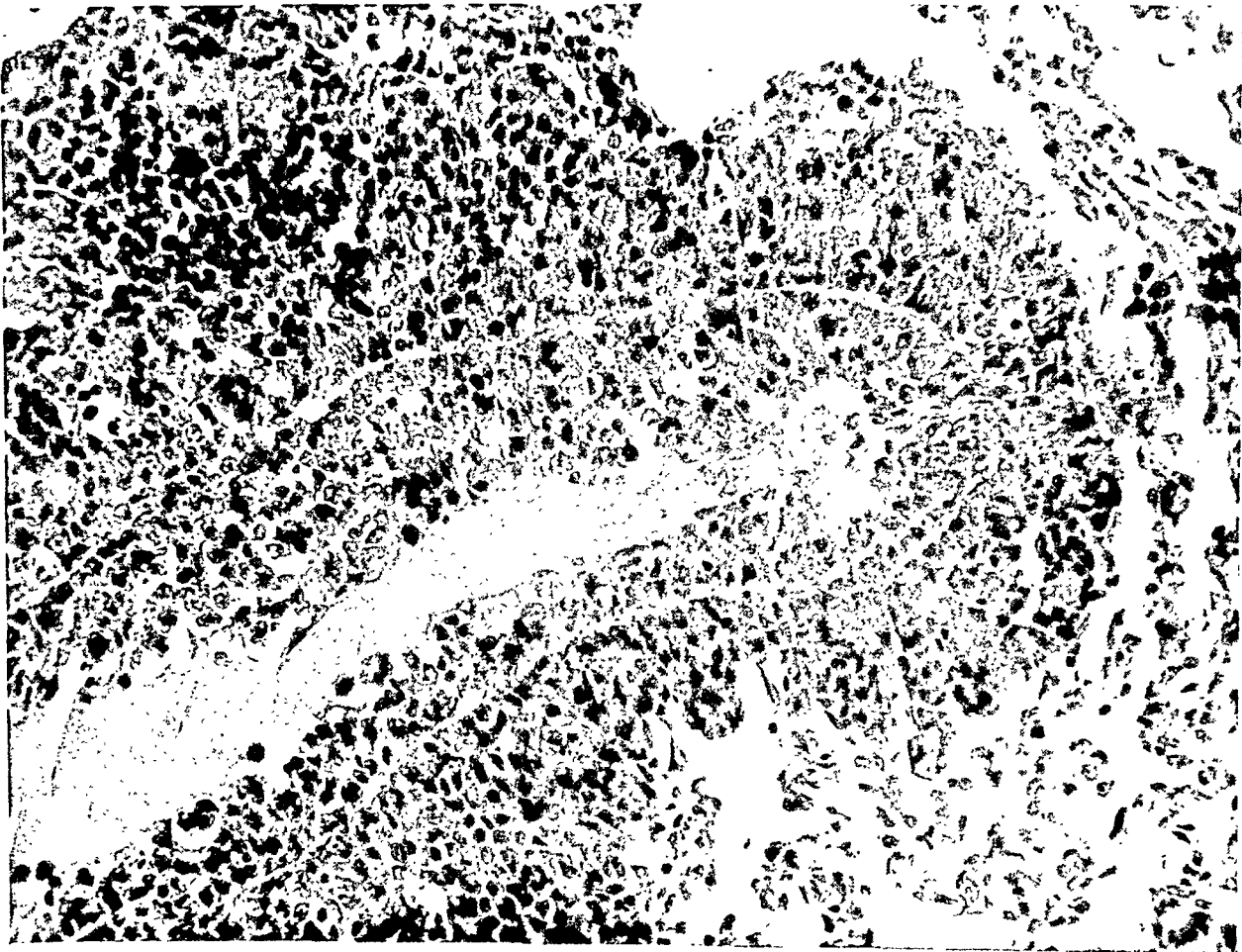
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## THE MORPHOLOGY OF BAUXITE-FUME PNEUMOCONIOSIS \*

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A lung disease associated with the manufacture of alumina abrasives was uncovered recently through an industrial survey by Shaver and Riddell.<sup>1,2</sup> The manufacture of these abrasive powders, the principal ingredient being Arkansas bauxite, was greatly increased in the later years of World War II, and resulted in a new and lethal pulmonary hazard with distinct clinical, radiologic, and morphologic findings. The salient features of the morbid anatomy in 6 fatal cases are brought forward in this contribution. The industrial exposure, symptomatology, case histories, radiologic findings, and chemical assay are given briefly to complete the background of this new entity.

In the industrial process involved, an abrasive, made up of an aluminum oxide known as "corundum," is being produced. The processing is carried out in electric furnaces with the mix consisting of finely ground bauxite, iron, and coke. Carbon electrodes are lowered to the surface of the mix and fusion occurs at a temperature of 2000°C. Dense white fumes are evolved during this process, leading to contamination of the furnace rooms. All fatal cases have occurred in furnace feeders or crane operators.

The clinical syndrome common to all is shortness of breath, cyanosis, substernal discomfort, and recurrent episodes of spontaneous pneumothorax from rupture of emphysematous bullae. Percussion and auscultation of the chest reveal a variety of signs dependent upon the underlying parenchymal changes and the presence or absence of air in the pleural cavities. The radiologic evidence is so startling and different from that in other pneumoconiotic films that Shaver<sup>1</sup> characterized this dust disease as individual and distinct from the more common silicosis. Diffuse, irregular, lace-like, and granular shadows, greatly increased width of mediastinum, and collapse of the lung are the cardinal features of the roentgenogram.

The parenchymal fibrosis of the lungs is unlike anything previously encountered (Figs. 1 and 2).

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## REPORT OF CASES

## CASE I

H. B., a Norwegian, had spent the last 11 years of his life in the abrasive-manufacturing furnace rooms. The terminal 5 years of employment were as a crane operator above the charged steel pots. Past history, with reference to the lungs, revealed an attack of influenzal pneumonia in 1918, influenza in 1926, work in underground mines for 11 years, and intermittent rock drilling in a canal area for 12 years, but neither of these occupations entailed exposure to dusts high in silica.

*Gross Examination of the Lungs*

The lungs were heavy, indurated, puckered, and gray-black. The scar tissue in the interlobular septa gave a granular, pebbled appearance to the lung surface covered by thickened pleura. Pigmentation was heaviest in the regions of localized subpleural scars which extended downward into the underlying parenchyma. No pleural nodules were discernible. On section, the pulmonary tissue was pigmented. Wide regions of parenchyma had been replaced by irregular, sweeping strands and masses of dense, gray-black fibrous tissue. These masses of homogeneous scar were generally located midway between the hilum and the periphery of the lung and consisted of branching bands 2 to 4 cm. in width, with finer prolongations extending peripherally through the interlobular septa to the pleura and mesially along the walls of bronchi and blood vessels to the hilum. No discrete nodules were discovered. Between the larger areas of conglomerate fibrosis and the pleura were irregular zones of coarse emphysema. In a few areas, particularly toward the hilum, the parenchyma was gray-yellow, suggestive of pneumonic consolidation. The bronchi were of normal caliber and contained a grayish coagulum of mucoid material. The vessels showed varying degrees of perivascular scar thickening. All were patent. The tracheobronchial lymph nodes were slightly enlarged, firm, black, and without white, whorled nodules.

*Microscopic Description*

Microscopically, the fibrosis was diffuse, obliterated the normal architecture (Fig. 3), and was hyaline, with nothing to suggest its origin. Black pigment, obviously exogenous, was distributed in masses of charged phagocytes trapped between the heavy, swollen, collagen bands. No nodule formation as in silicosis was discovered, although the hyalinization of collagen simulated that condition to a certain extent. At the margins of larger masses the fibrous tissue extended into the septa between alveoli, greatly thickening their walls and encroaching upon the air spaces. The lung parenchyma showed marked septal swelling due

to intracellular edema and numerous young fibroblasts. There was irregular focal deposition of eosinophilic collagen. In some regions the acellular fibrotic tissue contained trapped lymphoid knots and dilated capillaries. The dense abutments of fibrous tissue were covered with stretched septal cells. Dilated emphysematous sacs were caught between the dusky hyaline strands of fibrous tissue. Pyknotic alveolar cells were trapped in the bundles of dense scar tissue. The alveolar spaces contained macrophages filled with black particles, numerous giant cells surrounding empty crystal clefts, and considerable cellular debris. Small isolated groups of alveoli revealed evidence of an acute pneumonia, but this feature was never pronounced. Dust cells were present in the foci of pneumonitis. The lung parenchyma in other regions was not remarkable except for alveolar capillary congestion.

The bronchi, surrounded by dense fibrous tissue, were filled with mucus and possessed a thickened submucosa, heavily infiltrated with lymphoid cells. The smaller bronchi caught in areas of fibrosis were distorted and filled with inspissated basophilic mucus (Fig. 4).

Arteries frequently revealed endarteritis obliterans, which involved chiefly the larger branches. Medial proliferation and hyalinization were the predominant vascular changes.

The tracheobronchial lymph nodes were remarkable chiefly for large accumulations of dust-filled phagocytes within the follicular structures. Scattered throughout the lymph nodes were minute patches of diffuse hyaline fibrosis, and a few of the lymph channels were lined with thin hyaline membranes. No silicotic nodules with redundant fibrosis were encountered.

## CASE 2

J. F., a white male, 42 years old, was a furnace feeder through the years 1940 to 1944. Antecedent history was of no significance. This man's death was hastened by an acute respiratory illness, 1 year after removal from his industrial occupation.

### *Gross Examination of the Lungs*

The surfaces of the lungs were covered by thickened pleura with remnants of adhesions. The parenchyma generally was replaced by heavily pigmented, diffuse, gray-black scar tissue without nodulation. Thick-walled bronchi and blood vessels traversed this diffuse scar tissue. In most places the fibrosis was so generalized that no crepitant lung remained. Near the periphery there were groups of dilated air sacs. Beneath the pleura in a few spots the tissue was soft, compressed, ragged, and markedly distorted, but the general appearance was that of collapsed bullous emphysema. At the bases of the lungs there was little parenchy-

mal distortion. There were numerous lobules of apparently acute pneumonia, red to yellow, in the basal gutters. The bronchial nodes were moderately enlarged, heavily pigmented, but not excessively hard. No trace of nodular fibrosis was detected.

### *Microscopic Description*

There were numerous large areas of conglomerate fibrosis seen on microscopic examination. These masses of dense scarring were strung out in a coarse network. Heavy strands of hyaline fibrous tissue radiated throughout the parenchyma. At their margins, where the architecture of the lung was still recognizable, alveolar walls were extremely thick and composed of hyaline tissue. Deposits of black dust were heaviest in the large masses of fibrotic material. A foreign body giant cell reaction surrounded lozenge-shaped, empty clefts which were principally intra-alveolar in distribution and associated with the polynuclear pneumonic reaction. Giant and foam cells were not found within the septa.

Widespread septal hyalinization was noteworthy. This intrinsic septal change compressed, distorted, and obliterated the capillary pathways and in some regions these lesions were grouped together to form cores of ramifying, dense fibrous tissue. Large, subpleural, emphysematous areas with delimiting borders of hyaline septal tissue were frequent.

### CASE 3

P. H., a white male, 33 years of age, had been a furnace feeder for 3 years. Past history was irrelevant. In the last year of employment there was a progressive increase of dyspnea, cyanosis, and sporadic incapacitating episodes of spontaneous pneumothorax. His condition rapidly deteriorated and death ensued in November, 1943.

### *Gross Examination of the Lungs*

The lungs were similar in outline, weight, and tissue texture to those described in cases 1 and 2. They weighed 450 gm. each, and were dark with diffuse, rubbery texture. Pleural thickening was marked, with adhesions in the interlobar fissures. Innumerable emphysematous blebs were noted over the upper portions. The lungs cut with increased resistance. Dense bands of scar tissue were found, particularly in the upper portions, fanning out to the periphery. The bronchial tree and blood vessels were negative. The hilar lymph nodes were negative.

### *Microscopic Description*

Microscopically, an important feature of the parenchyma was the distortion of the alveolar walls with an intense mononuclear and lymphocytic infiltrate along with swollen alveolar lining cells (Fig. 5). This

inflammatory septal lesion frequently was found between dense bands of old fibrous tissue which formed suspensory strands. The increased collagen was pericapillary in many of the affected septa. In some areas the collagen formed an eosinophilic cast of the septa; in other regions the septa were broad with a loose reticular and lymphoid stroma covered by swollen cuboidal alveolar cells. The lung parenchyma was distorted by giant emphysematous pockets arranged either in a fragmented cystic manner or as a latticework. These foci were lined with a dense fibrotic layer. Vessels, bronchi, and lymph nodes were negative.

#### CASE 4

R. L., a white male, 61 years old, had been a foreman in the alumina abrasive manufacturing plant for 15 years. For the last 4 years this man had worked as a furnace feeder. Previous to World War II, radiologic examination of his chest had revealed increased bronchovascular markings and some fine streaks of shadowing, but no specific diagnosis was made. This worker was symptom-free prior to 1941. His condition deteriorated progressively due to increased respiratory difficulty. He was totally incapacitated for the last 2 years of life and died in 1945.

#### *Gross Examination of the Lungs*

The previously fixed lung specimens were received in two portions, both resembling irregular blocks of black India rubber. The consistency was firm throughout. In the peripheral part of one region there were many dilated, ramifying bronchi measuring up to 0.6 cm. in diameter. A few subpleural, caseous, white foci were noted up to 0.1 cm. in diameter. Projecting from the medial and basal portions of the lungs were a number of emphysematous bullae measuring up to 2.0 cm. in diameter. The pleural surface was opaque, granular, and in some places had tough, grayish white and stringy tag ends of adhesions. The granularity was particularly marked over the vertebral borders. The opacity and stringy adhesions were enhanced over the anterior borders. The bronchi and blood vessels were incarcerated in this noncrepitant, gray-black, resilient tissue.

#### *Microscopic Description*

Microscopically, there were four salient features in this lung: (1) Of cardinal importance was the fine, diffuse, septal fibrosis and marked hyalinization. This septal change probably represented the earliest lesion, and the agmination of these infiltrated hyalinized septa led to massive conglomerate fibrosis (Fig. 6). Extracellular carbon drifts and birefringent particles accompanied the massive scar tissue (Fig. 7). Cubical alveolar lining cells were a feature of the secondary parenchymal changes. This reaction was most intense in association with the dense, conglom-



erate fibrosis. Occasional giant cells arose from these cubical cells. (2) There was massive fibrosis as in the other cases. This diffuse, radiating fibrosis was old, in some places hyalinized. No amyloid was encountered. (3) A banal inflammatory infiltration of the alveolar septa by mononuclear and plasma cells was noted. In many regions this reaction was within the dense, broad bands of scar tissue. In many regions the septa showed stuffed, swollen, club-shaped outlines filled with a mixed chronic inflammatory infiltrate. (4) Evidence for tuberculosis was given by clear-cut histopathologic criteria, consisting of caseous foci, with surrounding giant cell systems. In these regions there was a definite attempt at a peripheral defense fibrosis with drifts of black pigment incorporated. The morphologic features of tuberculosis were dominant and virgin in these areas and certainly not modified as in the koniophthisis of Belt.<sup>3</sup> The shadows of scarred pulmonary parenchyma were still present despite caseation, which indicated tuberculosis to be a late sequel. Endobronchial tuberculosis was found also. Extreme emphysema was noted in some regions and endarteritis obliterans was noted in many of the vessels. Lymphoid follicles, free of reaction, were scattered throughout the lung. The bronchial lymph nodes showed reactive hyperplasia, minimal hyaline fibrosis in the littoral spaces, and freedom from nodule formation.

#### CASE 5

N. C., a white male, 41 years old, had been a furnace feeder for 6 years and a crane operator in the furnace room for 4 years. Past history was irrelevant. For a period of 1½ years after December, 1945, there was progressive increase in respiratory symptoms and signs accompanied by lace-like shadows in the radiologic chest plate. This workman died 2 years after removal from industrial exposure.

#### *Gross Examination of the Lungs*

The right lung weighed 675 gm., with the pleura adherent over the upper portion. The remaining pleural surface was opaque. Along the lateral border there were a number of blebs measuring up to 1.5 cm. in diameter. The entire lung was of homogeneous rubbery consistency and cut sections revealed a diffuse, indurated, gray to rusty brown color. Neither nodules nor any gross evidence of tuberculosis could be found. The bronchi and vessels were negative. The left lung weighed 620 gm. and was similar in all respects to the right lung. The lymph nodes in the hilar regions were soft, gray-black, and somewhat enlarged.

#### *Microscopic Description*

On microscopic examination, the entire lung parenchyma was stiffened by disseminated, variegated, septal scarring. Many of the alveolar walls

contained young fibroblasts and capillary buds, or showed spotty hyalination. In other regions fragmented, eosinophilic, collagenous material filled the alveolar walls. Overlying septal cells were cubical in areas and some alveolar lining cells were charged with coal-black refractile particles. In a few areas piling up of septal cells was noted; frequently buds were formed, filling the distorted alveolar spaces. The cuboidal septal cells often rested upon dusky fibrovascular buds. Retained secretion was frequently surrounded by cuboidal cells and occasionally by foreign body giant cells.

Reticulum, demonstrated by Laidlaw's method, was pericapillary in distribution in the least thickened of the alveolar septa. In regions of marked collagenous sclerosis there was still persistence of the alveolar pattern.

Thickening of the bronchial submucosa and a lymphocytic-eosinophilic inflammatory infiltrate were frequent findings. The vascular channels showed thickening of the outer media in the larger branches; the smaller showed endarteritis. The lymph nodes showed a preserved architecture, and no indications of silicosis were found.

#### CASE 6

R. M., a white male, 51 years old, had worked on the hot-change process for 4 years and then as a laborer in a winery for 1 year. Past history was otherwise non-contributory. Radiologic examination prior to his work as a laborer showed diffuse pulmonary shadows and bullous emphysema. One year later, a sudden pneumothorax developed with acute dyspnea and cyanosis, terminating in death.

#### *Gross Examination of the Lungs*

Microscopically, the left lung weighed 325 gm. and was greatly shrunk and compressed by a rough pleura, 0.4 cm. thick, which was plastered over the lower portions of the gray-black, indurated parenchyma. The cardinal feature was the presence of giant bullae around the periphery of the lung and emphysema in the upper portions. Some of these bullae reached a diameter of 4.0 to 5.0 cm. The lower part of one lung was firm and rubbery. The cut section revealed a uniform collapse of the parenchyma and a fish-net appearance in some regions. A few of the bronchi in the lower lobe were dilated.

The right lung weighed 375 gm. and was compressed throughout. It showed similar pronounced bullous formation. Cross section throughout the parenchyma revealed gray-black scarring with a rough, granular texture. The interlobar fissures were obliterated and replaced by a thickened scarred septum. Rough, gray-black, granular atrophy of the lung parenchyma was particularly prominent in the upper portions. Bronchi, vessels, and lymph nodes were negative.

*Microscopic Description*

Microscopically, the lung architecture in many regions was completely altered by diffuse interstitial fibrosis of a relatively mature type. The normal delicate filigree pattern was erased and the septa were thickened variably with fibroblasts and dense collagen. The septal fibrosis merged into irregular bands of old vascularized sclerotic tissue along the larger vessels and bronchi. This distribution was well demonstrated by the use of van Gieson's and Mallory's connective tissue stains. In many regions the interstitial fibrosis was sharply delineated and assumed many bizarre configurations like Chinese lettering. Smaller vascular channels showed thickening of their walls by collagenized tissue. Septal walls were broadened, stiffened, blunt-ended, and frequently were covered with hyperplastic cells. The septal lesions showed pronounced fibroblastic proliferation mingled with mononuclear cell infiltrate, and the broad, irregular bands of scar tissue were considered to be mats of such septal fibrotic lesions (Fig. 8). In the intertwined, spreading bands of old scar tissue and in the alveoli there were irregular deposits of black dust. Lymphoid follicles in the regions of interstitial fibrosis were intact. Reticulum, revealed by silver impregnation, lacked regular density and was arranged in a haphazard manner. In regions where septal fibrosis was not profound, alternating collapse and emphysematous pockets were found. Marginal emphysema was well advanced. The bronchioles within the scar reaction were dilated. A few showed squamous metaplastic changes and their lumina contained excessive secretion. Vascular channels showed thickening of their walls, narrowing of their lumina, and collagenous sclerosis outside the media of the vessels. The lymph nodes showed hyperplasia of the sinus reticulum and minimal amounts of fibrotic scarring of lymph sinusoids.

## CHEMICAL INVESTIGATION

The chemical approach to bauxite-fume pneumoconiosis offers a means of seeking the specific dust responsible for this unique pulmonary fibrosis. Investigation by chemical analysis is continuing under Dr. C. M. Jephcott, of the Division of Industrial Health, Ontario Department of Health. A few facts culled from the chemical analyses are presented in Tables I and II to offer a linkage with the described pathologic features.

Spectrographic determinations revealed that the majority of the fume particles are not greater than  $0.5\ \mu$  in diameter. Analysis by roentgen diffraction shows that the fumes consist mainly of amorphous material.

From Tables I and II it is evident that amorphous silica and alumina represent the two prominent constituents. Further work has shown that

spectrographic analyses of the ashed lung residue from the fatal cases give results similar to those of the furnace fumes, thus offering a close chemical correlation between cause and effect.

TABLE I  
*Analysis of Furnace Fumes*

Chemical	Range
Silica	29.0%—44.0%
Alumina	41.0%—62.0%

TABLE II  
*Chemical Analysis of Lungs*

Case	Silica in ash	Alumina in ash
1	30.5%	30.4%
2	24.8%	40.5%
3	25.3%	Not examined
4	21.2%	28.9%
5	28.6%	40.2%
6	31.0%	25.7%

### COMMENT

It is important to recapitulate the lung changes encountered in this entity. The specific pathologic findings were confined to the lungs. The outstanding features of the lungs from the gross examination were the relatively normal size and the gun-metal color. On palpation, the diffuse, widespread induration was of note. A fine fish-net pattern was present in some regions; elsewhere, radiating bands or masses of rubbery black tissue were more evident. Of further importance

was the absence of shotty or confluent nodulation. Only in case 4 were caseous foci of active tuberculosis encountered and these had not been altered by the diffuse scarring. The diffuse fibrosis in these lungs was probably responsible for the spreading out of the anthracotic pigment and accounts for their gray-blue color. The frequency and size of emphysematous vesicles were noteworthy. The bullae were not only found incarcerated within the fibrosed parenchyma but were especially prominent in the subpleural regions and frequently reached giant size. Tags of adhesions and pleural thickening were common, particularly over the upper lobes, but pleural thickening was frequently encountered wherever the lungs had been collapsed by repeated spontaneous pneumothorax.

The hilar and tracheobronchial glands were not enlarged or hard, bore the usual amount of anthracotic pigmentation, and were completely free of nodulation.

Histopathologic examination showed a constant pattern of diffuse fibrosis. It is our belief that the initial lesion was intracellular septal edema with early fibroblastic proliferation. The next salient feature was infiltration of inflammatory cells, principally lymphocytes and mononuclear cells within the thickened alveolar walls. The fibroblastic proliferation was succeeded by collagen deposition. The irregular "starched" trabeculae made up of hyalinized alveolar walls were seen to best advantage in the material from case 5. At this stage of the morphogenetic

process, fibrosing septal walls might remain distinct, giving the stiffened reticular pattern, or these alveolar walls might be matted together and produce wide bands of scar. The fibrous scar was eventually hyalinized, either focally or diffusely. Doubly refractile, particulate matter was frequently demonstrated by polaroid examination. Distribution of these particles was irregular and haphazard in these lungs. The sites of their deposition and the particle size and shape were not of a decisive nature.

Anthracotic pigment was present in varying amounts and in a variable pattern. Its usual distribution was often disturbed and in many regions the carbon pigment was incarcerated in the dense bands of scar tissue. Within the diffuse scar tissue, bronchiolectasia and dilated alveoli filled with basophilic, trapped mucus frequently were noted. Septal cells in these sites were cuboidal, this transformation being particularly frequent where the alveoli were incarcerated in bands of collagen. Squamous metaplasia at the bronchiolar level was uncommon and not present to the same degree as the occurrence of cuboidal cells. These cellular changes are not specific, being frequently noted in diverse chronic pulmonary conditions such as those resulting from bronchial obstruction, or from cadmium smoke poisoning.

Obliterative endarteritis was frequent and reached the greatest intensity in the regions of diffuse fibrosis. Collagenous sclerosis was particularly marked in the outer portions of the vessels. There was no invasion of the walls by pigment-laden macrophages.

Confluent bronchopneumonia, tuberculosis, and lipidosis were non-specific features found in individual cases. Acute infections are frequent but play no part in the progression of this disease. Foreign-body giant cells surrounding lozenge-shaped clefts were prominent in case 2 in the regions of pneumonic reaction. Tuberculosis in case 4 presented no special features. This pneumoconiotic background may offer a nidus for tubercle bacilli but the resulting reaction is not modified as in some other pneumoconioses, for example, siderosilicosis or the coal miner's lung. Alveolar bleb formation and emphysematous vesicles were prominent. This feature is apparently encountered relatively early in this disorder, as revealed by case 6. In the walls of some of the emphysematous blebs dense hyalinization was present. The fibrosis was pre-eminently in an interalveolar and interlobular pattern. Abundant septal scar tissue and lack of nodules are the characteristic parenchymal features in "alumina dust" fibrosis of the lung.

This tissue reaction bore no resemblance to any previously described process such as the acute interstitial fibrosis of Hamman and Rich.<sup>4</sup> The arrangement of the scar tissue is not that of carnifying pneumonitis of

infectious origin. There is no evidence of any specific infective granulomata or of sarcoid. Infarction and bronchial stenosis, either of neoplastic or inflammatory origin, could be eliminated in the consideration of these cases. In case 1, with a history of influenza, scars of an influenzal nature would not have persisted for the period of 25 years which elapsed before symptoms developed. No granulomatous reaction, such as that to beryllium, was present nor were asbestos bodies found; septal fibrosis is not encountered in these two lung disorders. None of the other dust diseases such as baritosis, bagassosis, or byssinosis need be considered because of the nature of the history.

All regional lymph nodes, both hilar and tracheobronchial, were free of specific inflammatory stigmata. There was neither diffuse nor concentric nodular fibrous tissue or hyaline material obliterating the lymph node architecture.

Due to the clear-cut industrial exposure and to the unique type of pulmonary fibrosis, it is accepted that this disorder occurring in workers in alumina abrasives manufacture is a dust-caused disease. The excessive, diffuse fibrosis throughout the lung tissue favors a "chemical dust" as the cause. The high silica content in the furnace fumes and in the lungs originally forced consideration of silica as the specific incitant, but this was abandoned for several reasons. Two principal objections are the existence of the silica in an amorphous form and the pattern of the pulmonary fibrosis. Only case 1 had a history of hard rock drilling, and there is no pathologic evidence of previous intense scarring from dust diseases. On morphologic grounds it is extremely difficult to believe that very finely divided vitreous silica or alumina could alter a pre-existent nodular fibrosis from rock-dust inhalation to a diffuse fibrosis.

The lungs in this pneumoconiosis lack the size, the nodulation, the configuration, and granite-like character of the silicotic lung. None of the indelible hallmarks of the silicotic reaction are present in any of these lungs. The lack of nodulation may be regarded as evidence against koniophages transmitting the inhaled particles of amorphous silica. The effect of this dust is primarily upon the septa, whereas in silicosis the original insult is borne by the lymphatics and lymphoid collections of the lung parenchyma. In this disease, the lymphoid collections are intact. Even in acute silicosis,<sup>5,6</sup> or in the burning of silica-bearing rock at Gaulley Bridge,<sup>20</sup> the histopathologic reaction in the lung is reminiscent of the silicotic pattern.

The polymorphism of silicotic fibrosis, due to diffuse fibrotic plaques, has recently been stressed by Costero.<sup>7</sup> The silicotic nodule in his cases assumed a diffuse pattern produced either by an organizing pneumonitis

or transformation of atelectatic zones into fibrous tissue. In the pneumoconiosis under discussion, neither of these features is encountered and the fibrosis in these cases is an autochthonous process.

It has been shown chemically that these lungs contain large quantities of amorphous alumina and silica. It is necessary to consider the part played by these two substances in the genesis of the fibrotic process. That silica and certain silicates are capable of producing nodular pulmonary fibrosis is well known. Gardner<sup>2</sup> has shown that extremely finely divided silica does not cause a diffuse fibrosis in the lungs of animals and that in most cases the silica is eliminated from the tissues too promptly to produce any lasting harm. Extremely fine silica is inhaled in an amorphous vitreous form from the "dense white fumes," but no human disease has as yet been traced to such finely divided silica. If this is a form of silicosis, the problem still remains to explain why the anatomic form of the reaction is diffuse, not nodular. It may be postulated that another dust or combination of dusts can be held responsible for the causation of this disease.

The admixture of dusts, such as hematite<sup>8</sup> or anthracotic pigment,<sup>3,19</sup> may modify a frank silicotic process but never completely erases it. King<sup>9</sup> recently has demonstrated that an antidotal mixture of aluminum hydroxide with quartz lowered solubility but did not prevent experimental silicosis. Hence if the aluminum dust combined with the amorphous silica is responsible for the production of this tissue pattern, it is evoking a fundamental biologic reaction of a previously unheralded type.

In Germany<sup>10-16</sup> cases have recently appeared when there was a greatly accelerated production of explosives in which only alumina dust was utilized. Apparently a clinical, radiologic, and morphologic disease was produced, similar to bauxite-fume pneumoconiosis. The German experiences with "aluminum dusty lung" have been referred to by Perry<sup>17</sup> in a recent report. A personal communication to one of us (A.C.R.R.) indicates the existence of a similar condition in Sweden.<sup>18</sup>

It is suggested, therefore, that the mechanism is that of an amorphous dust evoking a rapid, sclerosing process within the pulmonary septa and interfering with the koniophage transmission mechanism usually responsible for dust elimination. The end-result is a diffuse interstitial fibrosis with absence of nodule formation. We favor considering the amorphous alumina dust in intense concentration as the dominant etiologic agent, but the final answer as to whether these unusual morphologic lesions owe their development to the combined effect of amorphous silica and alumina in the fumes or to the alumina dust alone will have to be obtained from experimental, chemical, and pathologic studies now in progress.

Years ago, Cummins, quoted by Belt,<sup>3</sup> stated that the "lung was an oc-

cupational log book—it retains a qualitative and an indelible record of the mineral particles breathed during life and after death constitutes a sort of palimpsest of the industrial history.” With the emphasis on industrialization and the widespread use of diverse minerals, this statement has even greater significance. With new industries and accelerated manufacturing processes, new lung hazards will be followed by distinctive lung lesions.

It is our opinion, on the basis of the character of the pulmonary fibrosis and chemical analysis, that this disorder represents a distinctive pneumoconiosis.

### CONCLUSIONS

Pulmonary fibrosis has been found in 6 autopsies on workers in the alumina abrasive industry. The clinical course was one of short industrial exposure and rapid development of disease. The highly characteristic lung changes are those of diffuse non-nodular interstitial fibrosis frequently accompanied by emphysematous bullae. The exact cause has not yet been uncovered, but the hypothesis is that an intense exposure to amorphous aluminum dust may play a dominant rôle in this bizarre fibrosis.

Grateful acknowledgment is made to Dr. W. L. Donohue for his help with the photomicrographs.

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### DESCRIPTION OF PLATES

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#### PLATE 63

FIG. 1. Case 6. Collapsed, shrunken lung with widespread fine scarring. Large marginal bullae are seen. There is no evidence of nodulation.



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Wyatt and Riddell

Bauxite-Fume Pneumoconiosis

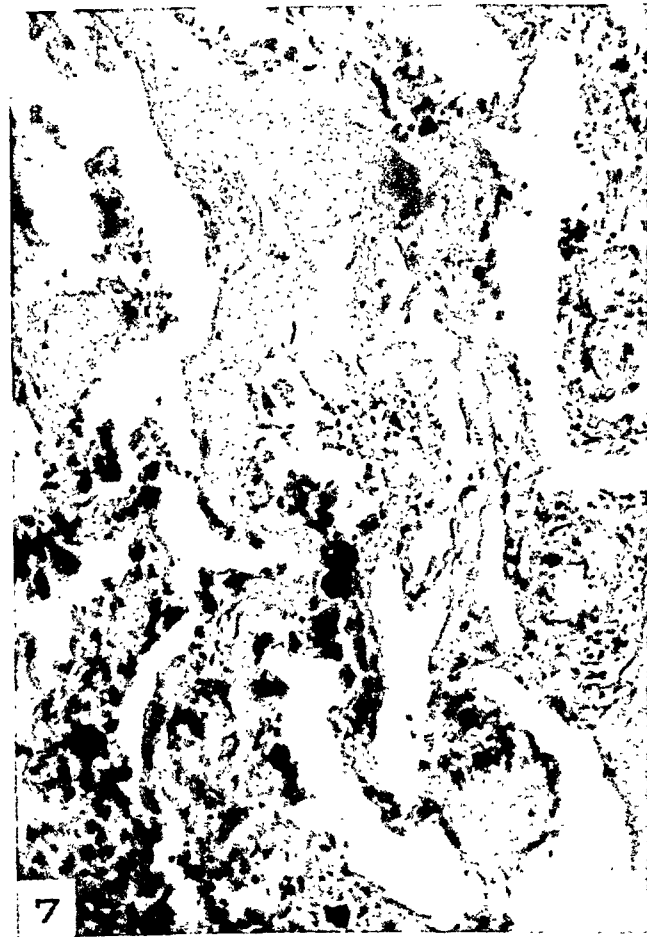
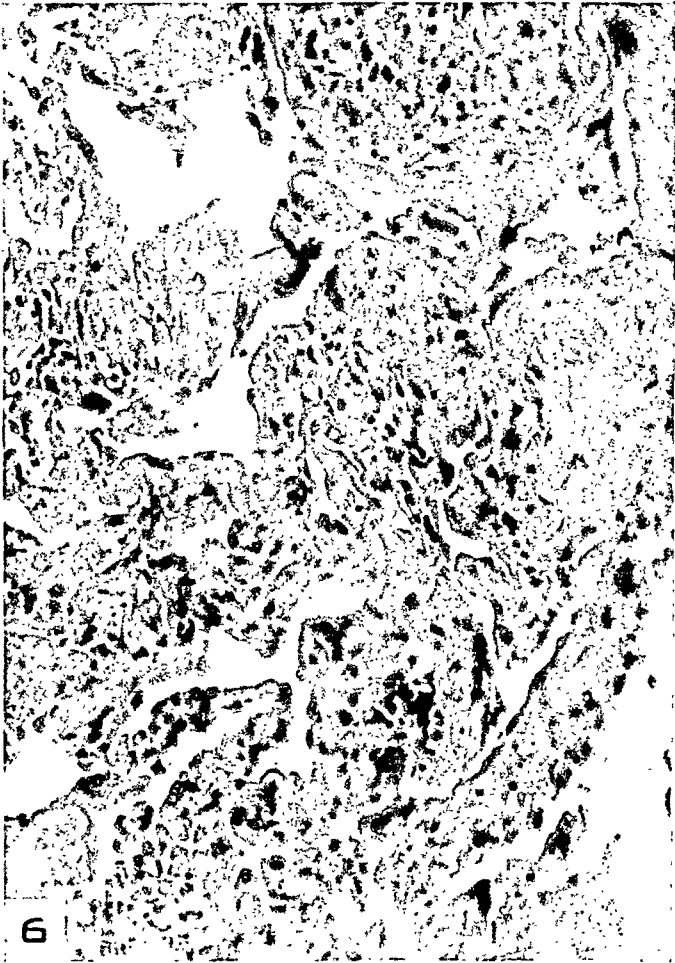
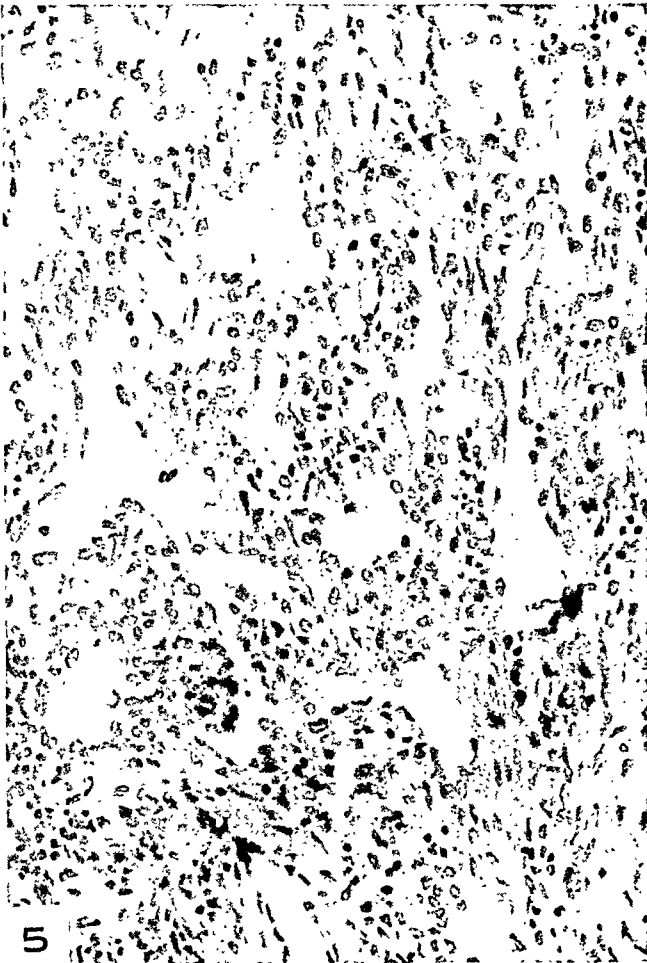
PLATE 64

- FIG. 2. Lung from case 4, showing a diffuse, black, rubbery parenchyma with trapped dilated bronchial spaces. There are no nodules in the parenchyma or lymph node.  $\times 2$ .
- FIG. 3. Case 1. Complete obliteration of the lung pattern by scar tissue and dilated air sacs. Scar tissue is accompanied by lymphocytes.  $\times 30$ .
- FIG. 4. Case 1. Distorted bronchial sacs filled with trapped mucus incarcerated by non-nodular fibrous tissue.  $\times 200$ .



PLATE 65

- FIG. 5. Case 3. Alveolar walls are swollen and thickened by mononuclear cells and fibroblastic nuclei. Obliteration of the capillary pathway may be noted.  $\times 450$ .
- FIG. 6. Case 6. Matting together of swollen, partially collagenized, distorted septal walls.  $\times 450$ .
- FIG. 7. Case 4. Irregular collagenization and hyalinization of septa. No nodular configuration is noted.  $\times 200$ .
- FIG. 8. Case 6. Dense bands and streaks of old scar tissue surrounding emphysematous pockets.  $\times 120$ .





## HIBERNOMA, A SPECIAL FATTY TUMOR REPORT OF A CASE \*

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The thought that fat is a variable tissue composed of cells of distinct histogenesis capable of special function has been expressed by several investigators for nearly a century. In 1853 Toldt<sup>1</sup> stated that adipose tissue in mammals constitutes a specific organ, distinct from connective tissue. The controversy which followed this expressed concept has resolved itself into the reasonable viewpoint that at least some fat exists during primitive development as pre-adipose tissue, some of which remains in multiglobular form. Kölliker,<sup>2</sup> in 1856, proposed the term "primitive fat organ" which Bell<sup>3</sup> accepted as being the equivalent of pre-adipose tissue. Presumably some multilocular fat (brown fat) is a temporary or transitory phase of ordinary adipose tissue, while some persists as a definite permanent structure. The latter has been called "glandular" fat and is pertinent to the subject here presented (Toldt,<sup>4</sup> 1870; Hammar,<sup>5</sup> 1895). This subject was well reviewed, summarized, and presented by Shattock<sup>6</sup> in 1908 and by Wells<sup>7</sup> in 1940.

The presence of gland-like structures composed of multiloculated fat cells in hibernating animals and some nonhibernating rodents was first discovered by Velsch<sup>8</sup> in 1670, and since then has been studied by several workers (Cramer,<sup>9</sup> Hatai,<sup>10</sup> Vignes,<sup>11</sup> Inglis<sup>12</sup>). Various names have been employed for these structures, including adipose gland (Rudolphi,<sup>13</sup> 1830), brown adipose tissue (Hammar, 1895<sup>5</sup>), oil gland (Jones, 1852<sup>14</sup>), lipoid or cholesterin gland (Cramer, 1920<sup>9</sup>), interscapular gland (Hatai, 1902<sup>10</sup>), organ of hibernation or hibernial fat (Vignes, 1913<sup>11</sup>), and hibernating gland (Barkow, 1846<sup>15</sup>). The word "hibernating" has been objected to on the grounds that it is not present in all hibernating animals. Auerbach,<sup>16</sup> in 1902, reported his study of this organ in 26 different species; altogether, 55 species have been studied by over 50 investigators.<sup>17</sup> The relationship of this variety of adipose tissue to the hibernating gland of the woodchuck has been studied by Rasmussen,<sup>17</sup> whose presentation in 1923 brought the knowledge of this structure up to date and gave credit to the contributions of earlier workers (Text-Figs. 1 to 3). That the hibernating gland develops in relationship to the thymus, a view held by some earlier workers (Velsch, 1670<sup>8</sup>;

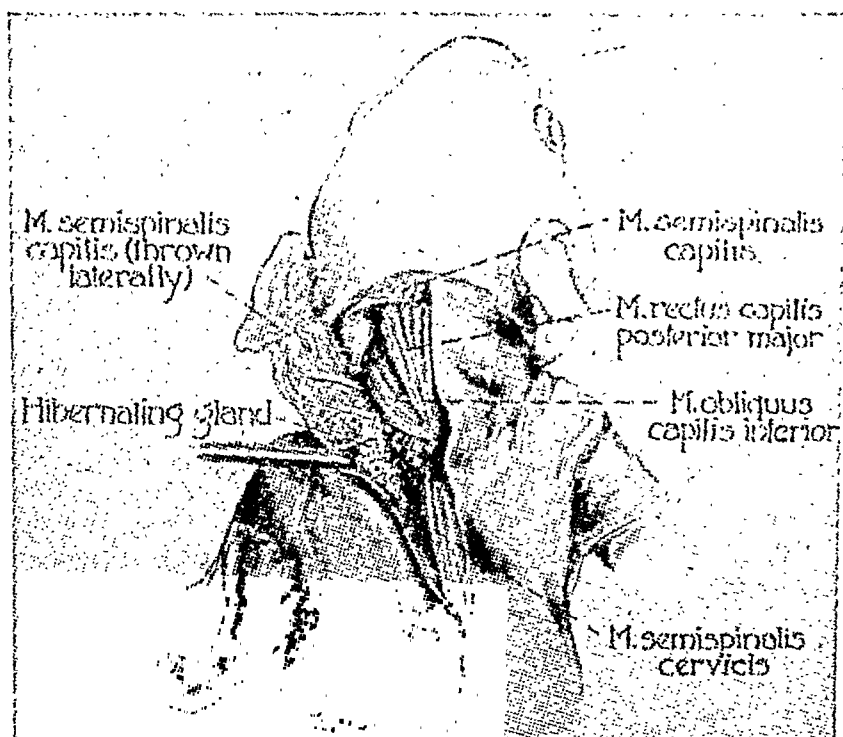
\* Presented at the Forty-Fifth Annual Meeting of The American Association of Pathologists and Bacteriologists, Philadelphia, March 12, 1948.

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Meckel, 1806<sup>18</sup>), has not been mentioned by later observers. The actual transformation of ordinary fat to brown fat in hibernating animals during lethargy probably does not occur.

The hibernating gland has been described as a paired, light brown, lobulated, richly vascular mass, somewhat resembling salivary gland or pancreas, composed of cells measuring from 25 to 50  $\mu$  in diameter which are coarsely granular or multiloculated and fat-containing. The cells are about half the diameter of those of ordinary adipose tissue. The amount

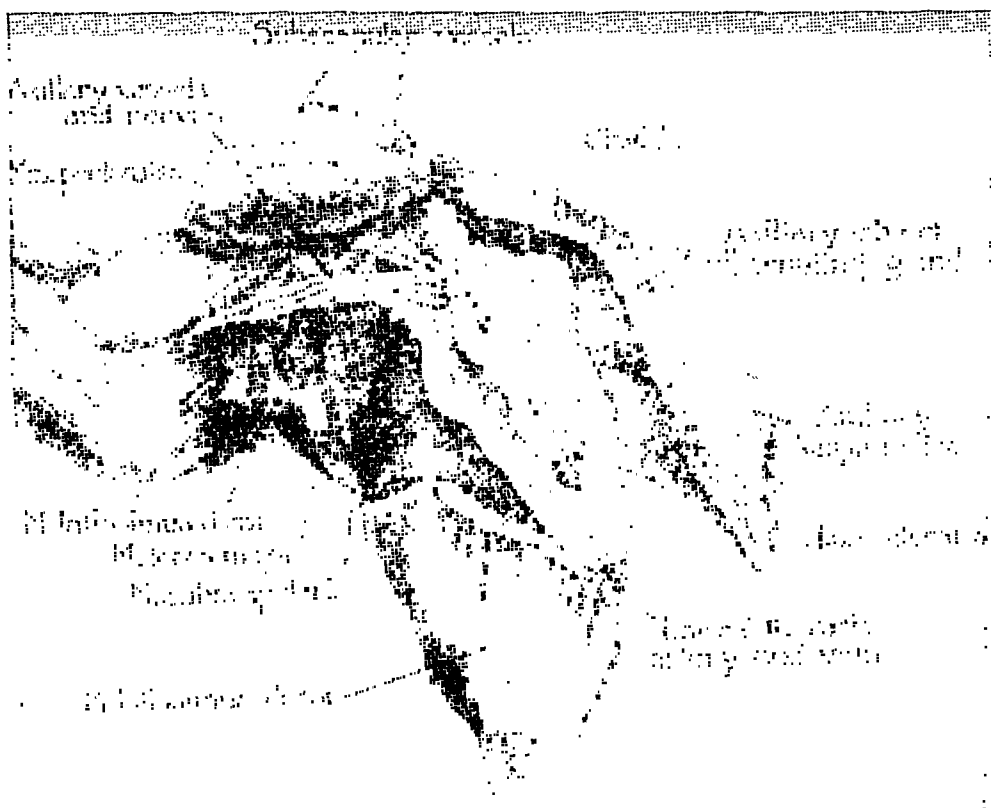


Text-Figure 1. Cervical portion of hibernating gland of adult woodchuck. (Reproduced by permission from *The Journal of Morphology*, 1923, 38, 147-205, as were also Text-Figures 2 and 3.)

of extractable fat in the hibernating gland is considerably less than that of ordinary adipose tissue (Carlier and Evans,<sup>10</sup> Shattock<sup>6</sup>). The lipid content is high (Shattock,<sup>6</sup> Vignes,<sup>11</sup> Cramer<sup>9</sup>). The pigment is apparently a lipochrome (Carlier and Evans<sup>10</sup>). Approximately 15 per cent of the gland is protein (Carlier and Evans<sup>10</sup>). Schoenheimer<sup>20</sup> (1942) has shown that lipids of the fat depots are not static or metabolically inert but are constantly subject to a variety of complex chemical reactions including synthesis, degradation, and interconversion. Fleischmann<sup>21</sup> stated that brown fat consumes more oxygen than white or yellow fat and Gomori<sup>22</sup> found that brown fat contained lipase whereas ordinary fat does not. Complete removal of this gland usually causes the death of the animal (Vignes<sup>11</sup>).

In hibernating animals, and some nonhibernating rodents, hibernal

masses have been found in the cervical region, axillae, between the scapulae and about the kidneys, and in the lumbar, inguinal, and gluteal regions (Hammar,<sup>5</sup> Auerbach<sup>16</sup>), all of which are presumably extensions from a large primary mass in the superior mediastinum. Homologous masses of adipose tissue in humans have been described by Merkel,<sup>23</sup> Hatai,<sup>10</sup> and Shattock.<sup>6</sup> Bonnot<sup>24</sup> studied the interscapular gland in human embryos from 3.2 to 28 cm. and also in newborn human infants and in adults, and stated that its length may be one-quarter that of the

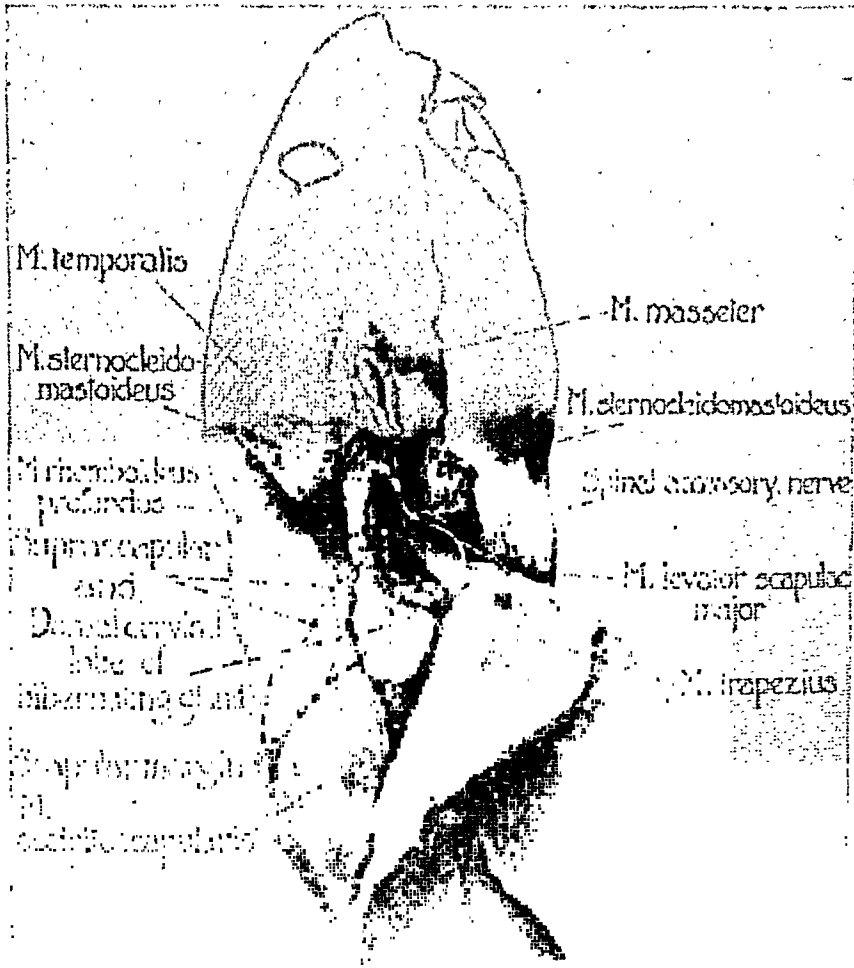


Text-Figure 2. Axillary lobe of hibernating gland of adult woodchuck.

crown-heel measurement of fetuses. Hatai<sup>10</sup> found the length of the gland to be up to 36 per cent of the length of the entire body.

The similarity of hibernating gland cells to the multilocular cells of developing (embryonic, immature, or juvenile) fat, sebaceous glands, and human brown fat (seen in cachexia or emaciation) is recognized. Neoplasms composed of incompletely differentiated fat cells, sometimes classified as atypical lipomas or liposarcomas, must be distinguished from hibernating gland tumors, the cells of which are not to be confused with immature fat cells sometimes encountered in lipomas. Whether a reversible transmutation can occur between the cells of the hibernating gland and ordinary fat is controversial. Hammar<sup>5</sup> was unable to produce unilocular fat cells in the hibernating gland by overfeeding rats. Hansemann<sup>25</sup> thought there were embryologic differences between ordi-

nary adipose tissue and hibernating gland and stated that one type does not change into the other. The possibility of these cells containing fine protein granules (Altmann's granules) from which fat may be synthesized has been expressed. Metzner<sup>26</sup> attempted to show that these granules are directly transformed into fat, but Bell<sup>3</sup> thought his observations



Text-Figure 3. Dorsal cervical part of developing hibernating gland in young woodchuck.

were far from convincing. Cramer<sup>9</sup> observed that these small granules increase in size and tend to push the nucleus to one side of the cell and to coalesce into one large drop. It is probably significant that the droplets and the granules are in the same arrangement. That the so-called hibernating gland is not a gland in the strict sense is realized, but its rôle as a possible source of internal secretion has been discussed. The designation "interscapular" is also probably unfortunate because it appears that only a small portion or none of the structure may actually lie between the scapulae.

In the development of the hibernating gland, a lymphoid and a vascular component have been mentioned. Hatai<sup>10</sup> stated that the "gland is composed of two entirely different constituent tissues; an outer fat tissue and an inner lymphoid structure . . . which contains an abundant supply of blood and lymph sinuses and vessels." He interpreted the inner portion as a hemolymph node. Two of the reported neoplasms of this structure have been cystic and vascular, suggestive of cystic lymphangioma. The significance of these two components in relationship to tumor formation is suggestive but not clear.

The first case of neoplasm of the hibernating gland appears to have been reported in 1905 by Merkel,<sup>39</sup> who employed the term "adenoma of sebaceous gland." Merkel had been interested previously in fat of this type and had introduced the term "Fettpolster."<sup>23</sup> The reported cases of this tumor which are probably and possibly authentic are listed in Tables I and II. It is difficult to evaluate the meager descriptions of case reports, and therefore inaccuracies and injustices cannot be avoided. Rasor's<sup>27</sup> case in 1913 and Bonnel's<sup>28</sup> about 1 year later seem acceptable. Bonnel's case presentation was discussed by Louis Gery of the Institute of Anatomy at Strasbourg. In this discussion Gery reported another case and introduced the term "hibernoma," a name which has been accepted by later contributors. Gery, who did not independently report his case, compared the color of the tumor to that of the suprarenal gland and described the cells as fatty epithelioid cells.

TABLE I  
*Collected Cases of Hibernoma, Probably Authentic*

Author	Year	Remarks
1. Merkel <sup>39</sup>	1905	Adenoma, sebaceous gland
2. Rasor <sup>27</sup>	1913	Female, age 25; back
3. Bonnel <sup>28</sup>	1914	Female; axilla
4. Gery <sup>28</sup>	1914	
5. Shaw <sup>29</sup>	1921	In exhibit in London, 1921
6. Inglis <sup>12</sup>	1927	Male, age 31; interscapular
7. Rochat <sup>30</sup>	1939	
8. Rochat <sup>30</sup>	1939	
9. Mosto and Radice <sup>31</sup>	1942	Female, age 37; axilla

Case 5 in Table I was mentioned in the notes of the annual meeting (1921) of the British Medical Association<sup>29</sup> in which reference was made to an exhibit arranged at Newcastle by A. D. Bernard Shaw containing a case from West London Hospital of lipoma glandulare (Inglis<sup>12</sup> also used this term), resembling hibernating gland and "thought to arise from a vestigial hibernating gland occasionally present in the human being." Rasor's<sup>27</sup> case report was combined with a contribution to the

granular synthesis of fat. The tumor which he reported was unusual in that it was a milky-white, soft, fluctuant neoplasm of the back and was composed to a considerable extent of unilocular fat cells, exemplifying all stages of transition between embryonal and adult fat. Reference to Rochat's<sup>30</sup> 2 cases appears in the minutes of the meeting of the Pathological Anatomical Society of the Netherlands held at The Hague in June, 1939. Rochat stated that he had had the opportunity to see 2 such cases which bore a perfect histologic resemblance to the hibernating organ of Inglis and that these have been called hibernomas. It is assumed that these were original cases not reported elsewhere. The anatomic sites of these two tumors were not stated. The title of the article by Mosto and Radice<sup>31</sup> was *Hibernoma de Gery*.

The cases which are included in Table II as possibly authentic are even more difficult to evaluate. Cases 1, 2, 3, and 5 were called sarcomas by the authors who reported them. The locations of cases 2, 3, 4, and 6 were not given. Camolle<sup>33</sup> "observed" 2 cases which perhaps should have been listed in Table I. They were studied by Uggeri,<sup>34</sup> who considered them to be similar to the cases of Rasor<sup>27</sup> and Merkel<sup>30</sup> (cases 1 and 2, Table I). Uggeri's case was placed in Table II because it was situated in

TABLE II  
*Collected Cases of Hibernoma, Possibly Authentic*

Author	Year	Remarks
1. Mallory <sup>32</sup>	1918	Female; back; lipoblastoma; malignant?
2. Camolle <sup>33</sup>	1921	Observed 2 cases. Original(?). No description. Cited by Mosto and Radice. <sup>31</sup> Lipoblastic sarcoma. Studied by Uggeri. <sup>34</sup> Similar to cases of Rasor <sup>27</sup> and Merkel. <sup>23</sup>
3. Camolle <sup>33</sup>	1921	
4. Inglis <sup>12</sup>	1927	
5. Börst <sup>36</sup>		Female, age 6 wks.; hygroma with glandular fat areas
6. Adair, Pack, and Farrior <sup>37</sup>	1932	Lipoblastic sarcoma, gluteal (noninfiltrating; nonmalignant)
7. Uggeri <sup>34</sup>	1938	Male, age 45; foam-celled tumor; xanthomyxolipoma
		Female, age 57; leg, atypical lipoma

the leg; the hibernating gland has not been described in the leg or thigh. Inglis'<sup>12</sup> second case (case 4, Table II) might have been a composite tumor composed in part of multilocular fat and may have been related histogenically to the hibernating gland. Perhaps some of these tumors could best be called atypical lipomas, as suggested by Uggeri,<sup>34</sup> or pseudo-lipomas, as suggested by Lunghetti.<sup>35</sup>

The histologic similarity of hibernoma to granular cell myoblastoma

should be mentioned because it is reasonably possible that some of the former have been mistaken for the latter. Such possible confusion could be eliminated by the employment of fat stains on all soft tissue tumors composed of granular cells.

### REPORT OF CASE

A Negress, 18 years old, was admitted (no. 46-11622) to the general surgical service of Detroit Receiving Hospital on September 9, 1946, because of a tumor of the right scapular region. She had been aware of a small lump in that region since early childhood but it had caused her no inconvenience until 1 year prior to admission. At that time the swelling had increased sufficiently to produce an unsightly bulge which could be seen easily through her clothing. The tumor had remained nontender at all times and the general health of the patient was good.

The past history was irrelevant and no other member of her family had recognizable tumors.

On admission the patient's temperature was 99° F.; pulse, 80; blood pressure, 135/80 mm. of Hg. Routine examination of the head, neck, chest, breasts, abdomen, and genitalia was noncontributory. Surgical scars were not present. Examination of the right scapular region revealed a soft, ovoid mass, measuring 12 cm. in diameter and elevated to 5 cm., dorsal to the right scapula. The mass was freely movable, nontender, slightly lobulated, and superficial vessels could be seen beneath the skin surface. There was a noticeable increase in local skin temperature.

Radiographic examination of the area indicated that there was no attachment of the tumor to the underlying bone.

Laboratory findings were as follows: Serologic tests for syphilis, negative; hemoglobin, 10.5 gm. per 100 cc. of blood; leukocytes, 4500 per cmm., with 68 per cent polymorphonuclear neutrophils (64 per cent segmented and 4 per cent nonsegmented), and 32 per cent small lymphocytes. Upon routine chemical and microscopic examination the urine was normal.

Surgical excision of the tumor was accomplished with considerable difficulty because of excessive vascularity. The mass was intimately attached to the deep fascia and muscle. The postoperative course was uneventful.

Upon gross pathologic examination the specimen was found to be a partially encapsulated, soft, discoid mass measuring 10 by 8 by 5 cm. and weighing 315 gm. Small fatty tabs were adherent to the surface. The mass was composed of fairly uniform, parallel bundles measuring from 2 to 5 mm. in diameter, which were separated with ease. The color varied from yellowish to reddish brown. After fixation in formalin the color was grayish brown and then the appearance was that of boiled muscle.

Histologically, the tumor was composed of large cells, most of which varied from 20 to 50  $\mu$  in diameter, with centrally placed nuclei in which the chromatin was finely and coarsely distributed. There were 1 to 3 nucleoli per nucleus, but the nucleolar-nuclear ratio rarely exceeded 1:4. The cytoplasmic-nuclear ratio varied from 4:1 to 10:1. The smaller cells contained fine and coarse granules; the larger cells contained small vacuoles or locules. Both the granules and locules stained black with

osmic acid and both were sudanophilic. In some multiloculated cells there appeared to be condensation or coalescence of smaller into larger locules, and when this became extreme the nucleus was then eccentrically or peripherally placed. A few unilocular fat cells were present which measured from 50 to 100  $\mu$  in diameter and, while it might be argued that these represented extrinsic adipose tissue, there seemed to be evidence of a transition from multilocular to unilocular fat cells. The tumor was divided into lobules, within which there was a small amount of finely divided reticulum. In sections cut perpendicularly to the above-described bundles the lobules were separated by loose areolar tissue.

*Report on Fat Analysis.\** The tissue was dried in a vacuum oven at 45° to 50°C. for 2 days. Drying was continued over calcium chloride for several days and the final weight was 27 per cent of the original specimen, indicating that the water content was approximately 73 per cent. The dried tissue was then extracted with alcohol and ether for 12 hours and this process was repeated several times. Sixty per cent of the dry weight was thus extracted. A portion of moist tissue specimen of similar size was refluxed with hot alcohol for 24 hours, the alcohol decanted into a weighed flask, and the dried residue weighed. This process was repeated several times with alcohol and then with ether until no fat was extracted and the remaining tissue was white and dry and could be ground and powdered. The extracted fat was 17.3 per cent of the net weight. The average of these determinations was 16.85 per cent, which represented the fat content of the tumor. The melting point of the fat was between 34° and 35°C. and the iodine number was 65.

The fat was then separated into the saponifiable and unsaponifiable fractions as described by Sperry and Bloor.<sup>38</sup> Of the total fat, over 80 per cent was saponifiable and 12 per cent unsaponifiable.

#### SUMMARY

The development of the so-called hibernating gland from persistent and permanent brown multilocular fat has been firmly established. Neoplasms developing in a homologous structure in humans have been occasionally reported since 1905 and now constitute an oncologic entity. The reported cases of hibernoma, by whatever name they have been designated, are difficult to evaluate, but 9 seem to be reasonably authentic, while 7 others are possibly acceptable.

The histologic study of the neoplasm in the case reported indicates that there is a transition from the "granular" cells to multiloculated cells

\* Performed by Ruth M. Davis, Ph.D., Department of Physiological Chemistry, Wayne University.

and even to unilocular fat cells. The fine granules sometimes referred to as Altmann's granules in the smaller neoplastic cells are apparently minute droplets of fat.

Care should be taken not to confuse this neoplasm with atypical lipomas, low-grade liposarcomas, or granular cell myoblastomas.

While "hibernoma" is admittedly a somewhat undesirable term, it possesses priority, for Gery, in 1914, gave an otherwise nameless tumor this name. The term refers not to hibernation but to the relationship of the neoplasm to hibernial fat. An attempt to find a more descriptive or appropriate name might easily lead to cumbersome terms and confusion. A challenge obviously exists for someone with a flair for terminology to improve or remedy this weakness in nomenclature.

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## DESCRIPTION OF PLATES

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### PLATE 66

FIG. 1. Hibernoma. External and cut surface of the gross specimen. The tumor measured 10 by 8 by 5 cm.

FIG. 2. Photomicrograph illustrating the lobulated architecture of the hibernoma. Hematoxylin and eosin stain.  $\times 200$ .

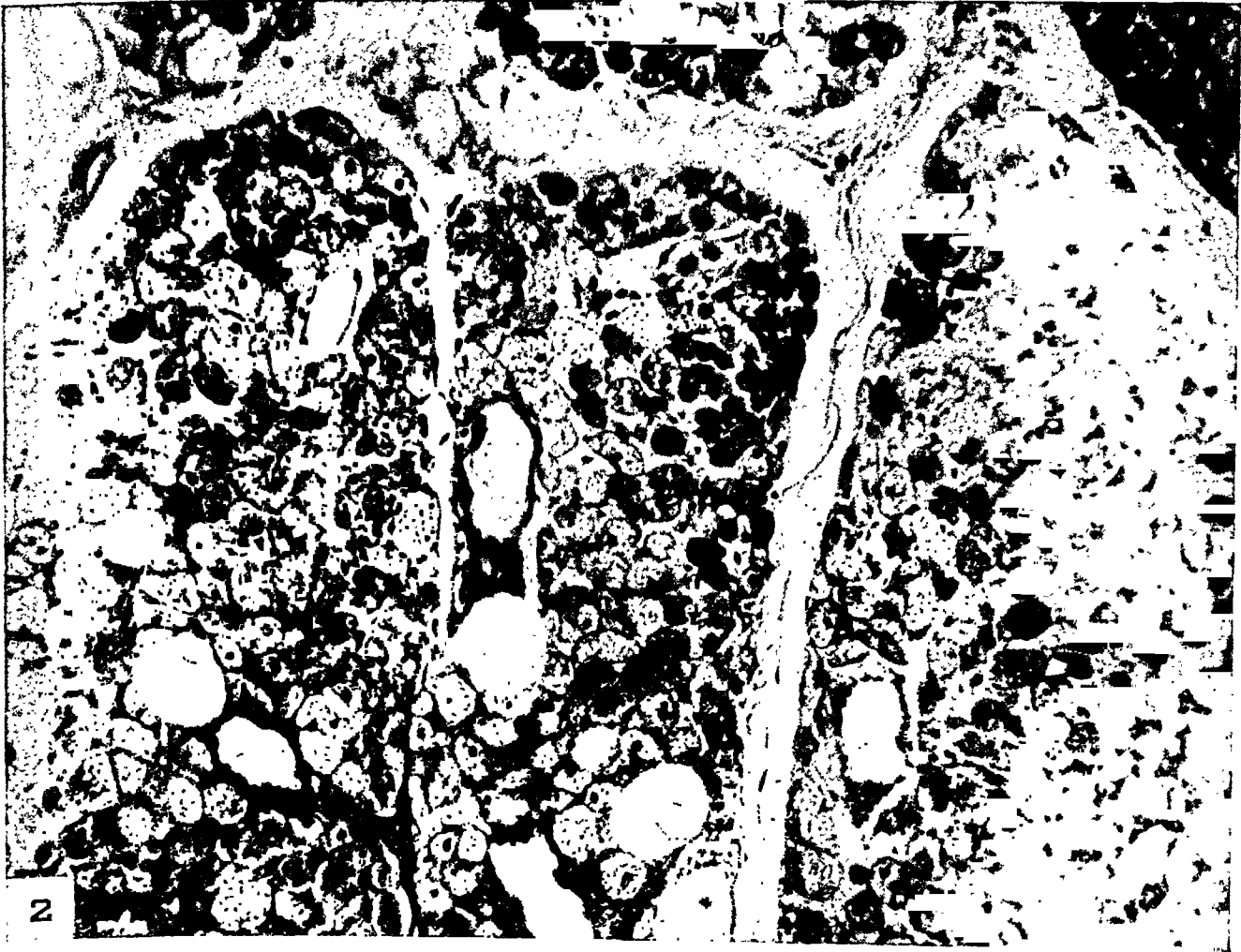
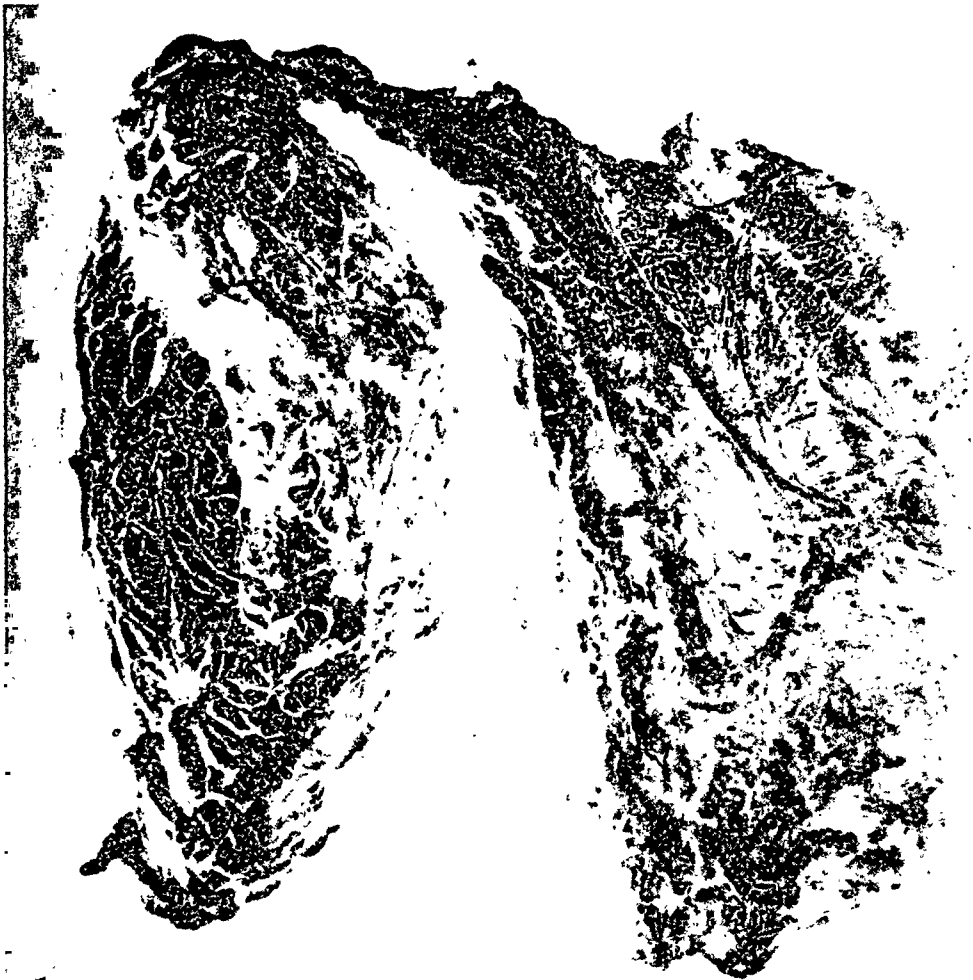
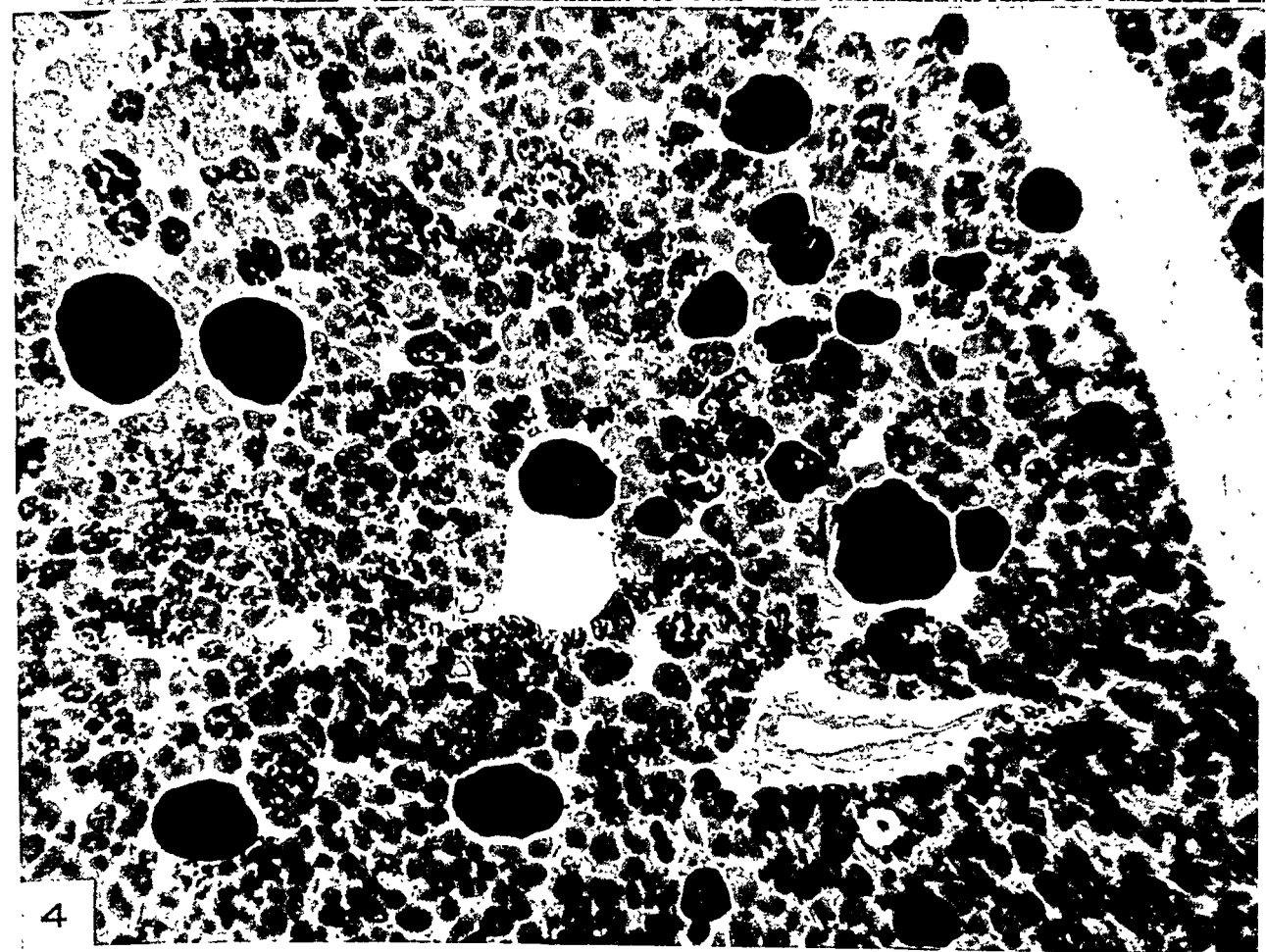
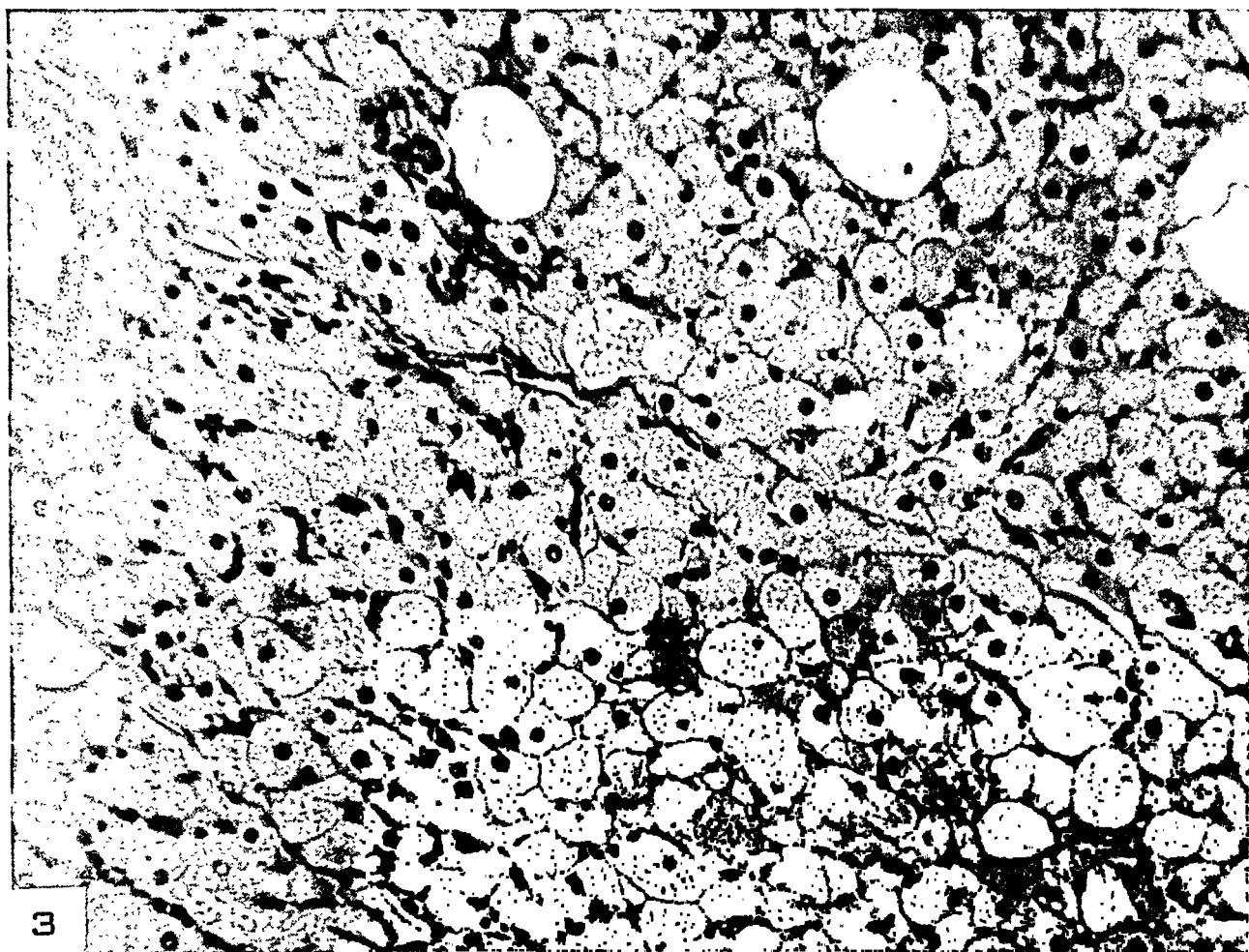


PLATE 67

FIG. 3. Cell size depends upon the fat content of the cytoplasm. Transition from finely granular cells, through multiloculated cells, and finally to adult fat cells is indicated. Masson's stain.  $\times 300$ .

FIG. 4. The varying degrees of fat content, and the size and distribution of the locules of fat are indicated. The fine granules are fat-positive. Osmic acid stain.  $\times 200$ .





## ARTERIOSCLEROTIC LESIONS IN PYRIDOXINE-DEFICIENT MONKEYS \*

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Adaptation of the rhesus monkey (*Macaca mulatta*) to a synthetic diet has opened the way to a re-exploration of the metabolic and structural defects resulting from single vitamin deficiencies. Such studies are particularly desirable in a primate, with metabolic processes similar to those of man. We have reported briefly our observations on thiamine<sup>1-4</sup> and folic acid<sup>5,6</sup> deficiency.

During the past 2½ years we have undertaken a systematic study of pyridoxine deficiency in the rhesus monkey. Observations have been made on the blood picture, blood and tissue contents of pyridoxine, and the metabolism of tryptophane, in addition to detailed morphologic examinations of tissues. This report is concerned with the pathologic findings, with particular reference to the degenerative and proliferative changes observed in arteries. A preliminary note of these observations recently has been made.<sup>7</sup>

### EXPERIMENTAL PROCEDURE

The basal diet was a modified M-3 diet<sup>8</sup> and contained powdered sucrose, 73 parts; vitamin-free casein, 18 parts; Hawk and Oser salt mixture, 4 parts; and corn oil, 2 parts. It was compressed in 2 gm. tablets following granulation and the addition of 1 per cent calcium stearate. The tablets were fed *ad libitum*. A tablet containing the following vitamin supplements was fed daily: nicotinic acid, 5 mg.; riboflavin, 1 mg.; thiamine chloride, 0.5 mg.; calcium pantothenate, 3 mg.; choline dihydrogen citrate, 100 mg.; paraminobenzoic acid, 100 mg.; inositol, 100 mg.; ascorbic acid, 25 mg., with sufficient powdered sugar to produce a tablet weighing 1.5 gm. In addition the monkeys received by mouth, twice weekly, 5 drops of vitamin A and D concentrate (100,000 international units of vitamin A and 10,000 international units of vitamin D per gm.), 385 µg. of pteroylglutamic acid,† 35 µg. of biotin, and 5 drops

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† Two of the monkeys were started on one-half this intake of pteroylglutamic acid. This is equivalent to a daily dose of 55 µg. and was originally reported to be adequate (Totter and associates.<sup>9</sup>). Dr. Totter later informed us that this intake was not optimal so we increased it to 110 µg. per day. Our own experience indicates that this is a more nearly optimal intake.

of mixed natural tocopherols\* once a week. Control monkeys also were provided with 3.5 mg. of pyridoxine hydrochloride twice a week.

Observations on 5 pyridoxine-deficient monkeys are reported in this study, 4 immature males and one female ranging in weight from 2375 to 3200 gm. During an initial control period the animals were given the diet with the complete vitamin supplement. In the absence of complicating influences such as infection, the animals ate the diet surprisingly well, gained weight, and remained vigorous and active. In fact, one monkey, maintained on the diet for 42 months, is one of the most vigorous, healthy monkeys we have had in the laboratory. After the control period, pyridoxine was removed from the diet. The animals ordinarily continued to eat well and gained weight for 2 to 3 weeks, after which the food consumption began to fall off and the animals lost weight. In most instances there was a gradual continued weight loss. Aside from the decreased food consumption, gradual weight loss, and diminished vigor the animals showed little change in appearance until the depletion had been carried for a period of 5 to 6 months. Then they became unkempt and somewhat sluggish unless disturbed, when they seemed hyper-irritable and "jumpy." Convulsions have not occurred. The hair usually became thinner and lighter; hair growth was almost completely arrested. Some animals showed edema of the eyelids and late in the course of the experiment most had developed some fissuring of the epithelium of the palms of the hands and feet. All developed a moderate leukopenia and anemia. Observations on the pyridoxine content of the blood indicated that this paralleled the intake. During the control period in which the animals received 1 mg. of pyridoxine daily, the pyridoxine values of the blood averaged 11  $\mu$ g. per 100 cc., while during depletion the values ranged from 1.5 to 4  $\mu$ g. per 100 cc.

#### PATHOLOGIC FINDINGS

To date, pathologic examinations have been made on 5 animals which had been subjected to pyridoxine deficiency for periods ranging from 5½ to 16 months. The anemia, leukopenia, and associated bone marrow alterations occurring in pyridoxine deficiency will be the subject of a separate report. Significant lesions were observed in the livers of 2 animals. One animal, subjected to pyridoxine deficiency for 16 months, showed a faun-colored, nodular liver in which degenerative changes and regenerative hyperplasia were evident. The other animal, deficient for 7 months, showed a mild periportal and diffuse fibrosis. It is evident that more extensive study will be required to determine the incidence and significance of this finding.

\* Nopco Chemical Co., Harrison, N. J.

This report is concerned primarily with the arterial lesions, which are the most constant and prominent abnormality encountered. Sclerotic lesions have developed in the arteries of each of the 5 animals subjected to pyridoxine deficiency. In the 2 animals which were examined at the end of 5½ months the changes, while definite, were not extensive. In one (R.47.441), intimal fibrosis was present in arterial branches in the pancreas (Fig. 1), kidney (Figs. 2 and 3), and serosa of the colon (Fig. 6). The other animal showed definite but minor changes in the branches of the renal arteries and in the coronary arteries. Similar lesions in the arteries of the kidneys and pancreas were seen in an animal subjected to deficiency for 7 months. More widespread and advanced arteriosclerotic lesions have developed in 2 animals which were maintained on a pyridoxine-deficient diet for 13 and 16 months, respectively. In these animals lesions were prominent in the coronary arteries (Figs. 7, 9, 10, 11, and 12), as well as in arteries of the kidney (Figs. 4 and 5), pancreas, and elsewhere. Particularly advanced changes were present in arteries of the testicle of one animal (Fig. 8). It was somewhat surprising to see an advanced "arteriosclerosis" in the vessels of an immature testis. Small plaques of fibrous tissue developed in the intima of the arteries mentioned, which bore a close resemblance to human lesions of arteriosclerosis. The new-formed fibrous tissue plaques lay between the endothelium and the internal elastic lamina. In some instances there were minute blister-like structures where the endothelium had separated from the underlying fibrous tissue (Figs. 9 and 10). Occasionally "splitting" and duplication of the internal elastic lamina occurred (Fig. 4). The lesions were well demonstrated by a combination of Weigert's elastic tissue and van Gieson's stains. They tended to be patchy in distribution (Fig. 11), a feature characteristic of arteriosclerosis in man. Occurrence of the lesions at bifurcation points (Fig. 12) was another feature of the experimental lesion common to the human disease. Lesions in branches of the renal vessels showed a relatively coarse, intimal, collagenous sclerosis. In the coronary vessels the intimal fibrosis consisted of a more delicate collagen and the plaques appeared more edematous. Detailed examination of the aorta was not made. Gross lesions were not noted, but microscopic patches of edematous intimal fibrosis of the type encountered in other vessels were demonstrated in the 2 animals maintained on the deficiency for over 1 year. Likewise, while detailed study of the blood vessels supplying the extremities was not made, analogous lesions were found in those vessels which were examined.

The pathogenesis of these experimental arteriosclerotic lesions has not been determined, but observations suggest that there is an alteration in the connective tissue ground substance of the intima. In sections



stained with hematoxylin, slightly basophilic, mucoid-appearing material has been seen lying between the proliferating connective tissue cells. This, with the "blister-like" separation of the endothelium seen in some instances, suggests some fault in a binding substance. This might be related to the defect in protein metabolism described in pyridoxine deficiency in other animals<sup>10</sup> and recently shown by us to occur in the monkey.<sup>11</sup> There seems no question that the arterial lesions are related to pyridoxine deficiency. Such lesions have not developed in monkeys on the same dietary regime but subjected to other dietary deficiencies and showing equal degrees of inanition. The experimental lesions which have been described have a close resemblance to arteriosclerosis as it occurs in man.

It is of interest to note that Virchow, according to Aschoff,<sup>12</sup> considered the first change in the atherosclerotic process to be a "certain loosening of the connective-tissue ground substance" of which the arterial intima is for the most part composed. "This swelling of the ground substance . . . is recognized microscopically by the increased width and homogeneity of the connective-tissue spaces." Aschoff adds, "Pari passu with the thickening and transformation of the ground substance, the connective-tissue cells of the intima undergo changes. They enlarge in all dimensions, divide and form localized thickenings." Other students of the disease have emphasized the fundamental proliferative aspect of the arteriosclerotic process. It would seem likely that imbibition of fluid and colloidal substances such as cholesterol from the blood plasma would be favored by such a pathologic process.

### DISCUSSION

Experimental attempts at production of arteriosclerotic lesions in primates have been notably unsuccessful. While Fox<sup>13</sup> has recorded the spontaneous occurrence of arteriosclerosis in certain captive primates, he has not observed the condition in *Macaca rhesus*. It is noteworthy that prolonged feeding of cholesterol has failed to produce significant lesions in the monkey.<sup>14</sup> This is, in fact, a potent argument against the cholesterol theory as a major influence in the pathogenesis of arteriosclerosis in man. As far as we know, the lesions here reported are the first of this type that have been described as resulting from deficiency of a specific food factor. The observation gains added significance in that the lesions have been produced in a primate which, in nutritional metabolism, is closely related to man. Furthermore, the lesions have resulted from an experimental circumstance that might occur in man.

While it has not yet been shown conclusively that pyridoxine is required by man, certain clinical observations strongly suggest that it is.<sup>15</sup>

It would be most surprising if this substance were not essential for normal metabolism in man inasmuch as it has been found to be required in the diet of all animals so far investigated.<sup>10</sup> Prior studies in animals have been concerned primarily with the influence of deficiency on the blood picture and on iron metabolism.<sup>10</sup> Vascular lesions have not been described heretofore. It is noteworthy that pyridoxine deficiency is in essence a chronic deficiency, relatively slow in evolution and without distinctive external manifestations. Such a deficiency state would be one particularly difficult of clinical recognition. It remains to be determined to what extent pyridoxine deficiency may occur in man and, if it does occur, what influence it may have in the pathogenesis of arteriosclerosis.

### SUMMARY

Pyridoxine deficiency in the rhesus monkey produces sclerotic lesions in arteries bearing close resemblance to those of arteriosclerosis in man.

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## DESCRIPTION OF PLATES

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### PLATE 68

All photomicrographs are of sections stained by a combination of Weigert's elastica and van Gieson's methods.

- FIG. 1. Intimal fibrosis in a branch of a pancreatic artery. Monkey R.47.441: pyridoxine deficiency, 5½ months.  $\times 162$ .
- FIG. 2. Early eccentric collagenous intimal thickening in a major branch of a renal artery. Monkey R.47.441: deficiency, 5½ months.  $\times 80$ .
- FIG. 3. Marked collagenous intimal thickening in a small branch of a renal artery. Monkey R.47.441: deficiency, 5½ months.  $\times 162$ .
- FIG. 4. Intimal collagenous sclerosis with reduplication of internal elastic lamina in a small branch of a renal artery. Monkey R.47.199: deficiency, 16 months.  $\times 325$ .

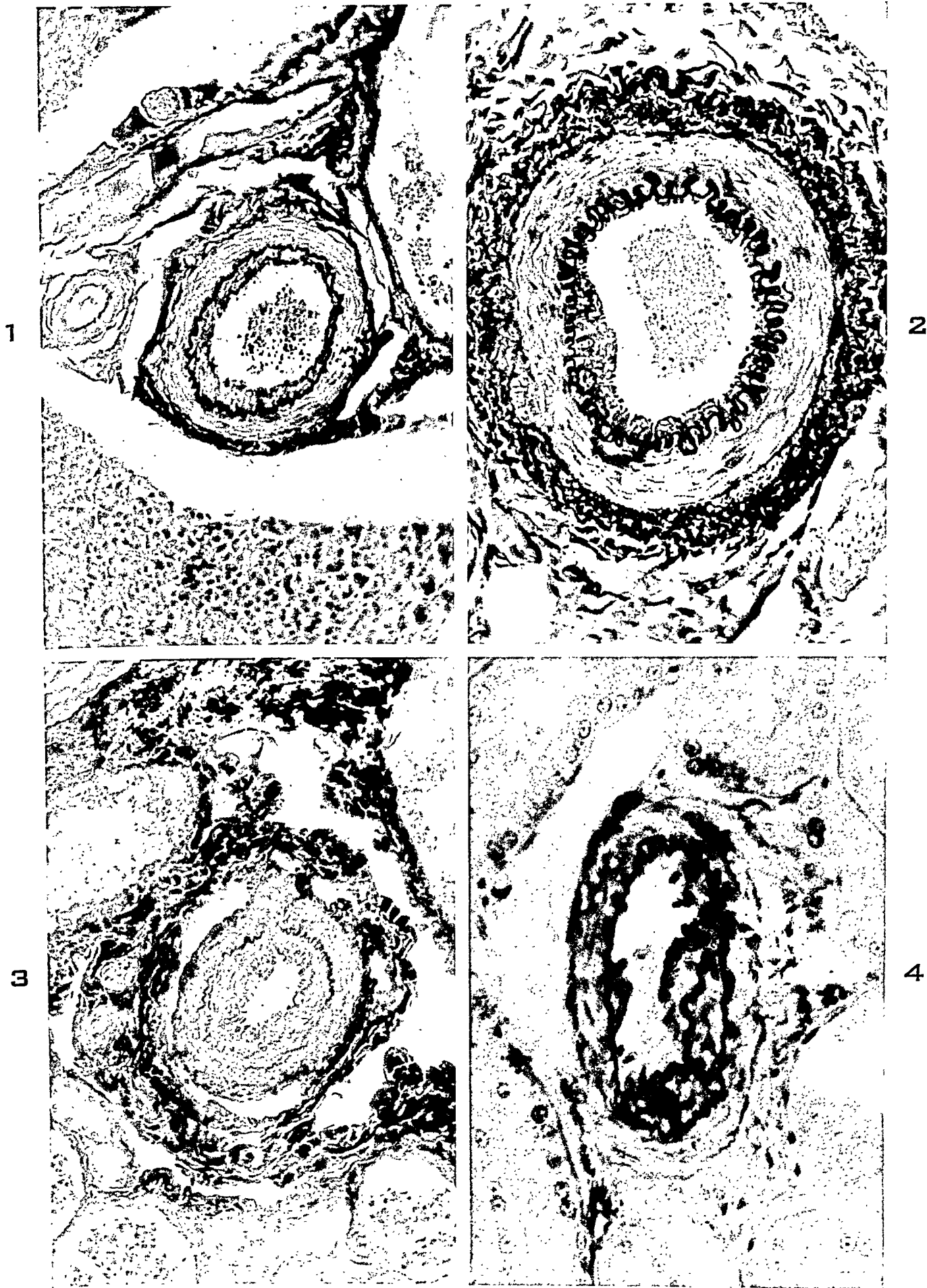


PLATE 69

FIG. 5. Intimal fibrosis in a small branch of a renal artery. Monkey R.47.1261: deficiency, 13 months.  $\times$  160.

FIG. 6. Uniform intimal fibrosis in small arteries in the serosa of the colon. Monkey R.47.441: deficiency, 5½ months.  $\times$  80.

FIG. 7. Early eccentric intimal fibrous plaque in a branch of a coronary artery. Monkey R.47.1261: deficiency, 13 months.  $\times$  160.

FIG. 8. Marked occlusive intimal fibrosis in an artery of the testicular tunic. Of note is the delicate edematous-appearing collagen. Monkey R.47.199: deficiency, 16 months.  $\times$  80.

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PLATE 70

- FIG. 9. Eccentric edematous hyperplasia of intimal fibrous tissue in a branch of a coronary artery. Monkey R.47.199: deficiency, 16 months.  $\times 162$ .
- FIG. 10. Major coronary artery showing marked hyperplasia of intimal fibrous tissue. Monkey R.47.199: deficiency, 16 months.  $\times 80$ .
- FIG. 11. Major coronary artery showing eccentric patches of intimal fibrosis. Monkey R.47.1261: deficiency, 13 months.  $\times 80$ .
- FIG. 12. Branch of a coronary artery at bifurcation zone. Of note is an evident predilection for fibrous intimal plaques at this site. Monkey R.47.1261: deficiency, 13 months.  $\times 80$ .

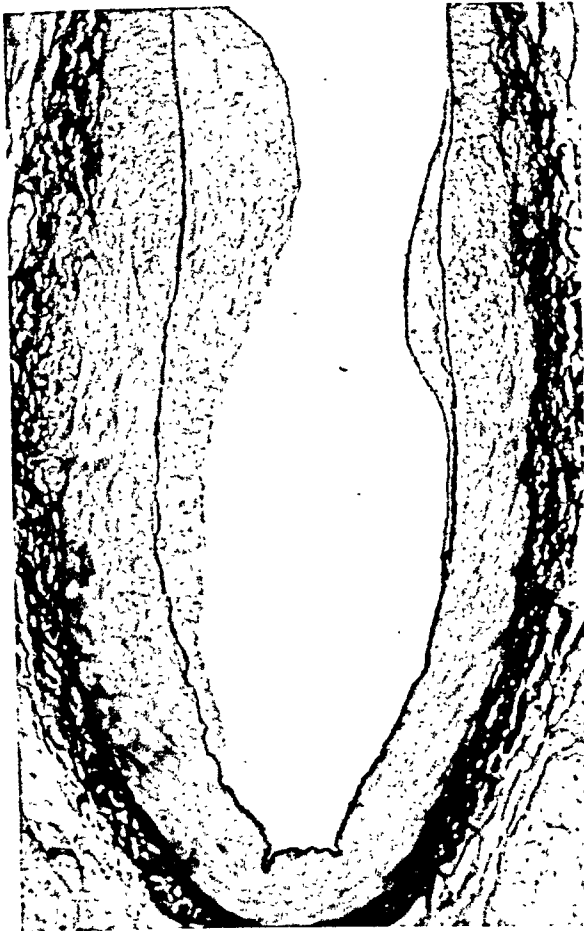
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# THE MORPHOLOGY, ANDROGENIC FUNCTION, HYPERPLASIA, AND TUMORS OF THE HUMAN OVARIAN HILUS CELLS \*

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The hilus of the human ovary contains nests of cells morphologically identical with testicular Leydig cells, and which, in all probability, produce androgens. Multiple sections through the ovarian hilus and mesovarium will reveal these small nests microscopically in at least 80 per cent of adult ovaries; probably in all adult ovaries if sufficient sections are made. Although they had been noted previously by a number of authors (Aichel,<sup>1</sup> Bucura,<sup>2</sup> and von Winiwarter<sup>3,4</sup>) who failed to recognize their significance, Berger,<sup>5-9</sup> in 1922 and in subsequent years, presented the first sound morphologic studies of the ovarian hilus cells. Nevertheless, there is comparatively little reference to these cells in the American medical literature, and they are not mentioned in standard textbooks of histology, gynecologic pathology, nor in monographs on ovarian tumors (with the exception of Selye's recent "Atlas of Ovarian Tumors"<sup>10</sup>).

The hilus cells are found in clusters along the length of the ovarian hilus and in the adjacent mesovarium. They are, almost without exception, found in contiguity with the nonmyelinated nerves of the hilus, often in intimate relationship to the abundant vascular and lymphatic spaces in this area. Cytologically, a point for point correspondence with the testicular Leydig cells can be established in terms of nuclear and cytoplasmic detail, lipids, lipochrome pigment, and crystalloids of Reinke.

This paper presents a morphologic study of the ovarian hilus cells, a report of 2 cases of masculinizing tumors of these cells, as well as 2 instances of hilus cell hyperplasia associated with masculinization. Additional data concerning the functional significance of the cells and their response to chorionic gonadotropin are recorded.

## TERMINOLOGY

A number of names have been applied to the hilus cells. Berger<sup>5-9</sup> referred to them as "sympathicotropic" cells. Kohn<sup>11</sup> used the term "extraglandular interstitial cells" or "extraglandular Leydig cells."

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Neumann,<sup>12</sup> Wieser,<sup>13</sup> and others have referred to them as "hilus cells."

The adjective "sympathicotropic" was applied by Berger because of the almost constant anatomic relationship to the nonmyelinated nerves of the ovarian hilus and mesovarium. However, the term has a physiologic connotation which is perhaps unwarranted in our present state of ignorance. The term "interstitial cell" is likely to be confusing. Interstitial cells of the ovary, apparently derived from theca interna, are prominent in certain animals but there is disagreement as to their existence in humans. In any case they appear unrelated to hilus cells. The term "extraglandular Leydig cell," though a bit cumbersome, has much to commend it. The cells are morphologically identical with Leydig cells and are undoubtedly androgenic. Nevertheless, absolute identity has not been proved; and it may be that the relationship to nerves indicates an additional function, not yet elucidated, which may establish the cells as cousins rather than sisters to the Leydig cells of the testis.

I shall refer to these cells as "ovarian hilus cells." Fortunately, none of the other numerous cell types resident in the ovarian hilus has been so designated. The term suffers, of course, from lack of specificity, but that perhaps may be an advantage at present.

#### RESUMÉ OF LITERATURE

There are scattered studies of ovarian hilus cells in the earlier literature by authors (Aschoff,<sup>14</sup> Bucura,<sup>2</sup> von Winiwarter,<sup>3</sup> and others) who considered them part of the chromaffin system. Since 1922, Berger,<sup>5-9</sup> in a series of studies, has presented convincing morphologic evidence of the similarity of these cells to testicular Leydig cells, referring to them as sympathicotropic cells. A lively controversy between the proponents of these two theories existed for many years. Reference may be made to the papers of de Winiwarter,<sup>15-17</sup> the stoutest proponent of the "paraganglion cell" theory, as well as to those of Wallart<sup>18</sup> and Joachimovits.<sup>19</sup> Since Berger's initial studies, most investigators have agreed with his conclusions (Kohn,<sup>11</sup> Pawlowski,<sup>20</sup> Brannon,<sup>21</sup> Wieser,<sup>13</sup> Barrozo do Amaral<sup>22</sup>).

Particular reference is made to the study by Kohn<sup>11</sup> (who, incidentally, discovered the pheochrome system). After a morphologic analysis he rejected the possibility that the ovarian hilus cell belongs to the chromaffin system. Most investigators, including myself, have been unable to demonstrate a chromaffin reaction in these cells. It is altogether likely that some who have reported chromaffin staining were misled by the brown lipochrome pigment normally present in many of the cells.

In the human newborn the identification of cells adjacent to the ovary is complicated, since true pheochrome cells also may be present, particularly in the broad ligament. Chromaffin tissue in the embryo, after separating from the sympathico-chromaffin anlagen, may be closely associated with the wolffian body. Zuckerkandl<sup>23</sup> noted that chromaffin tissue may be found in the fetus and newborn along the ureters, in the broad ligaments, retroperitoneal tissues, and elsewhere. However, these nests are morphologically distinct from the hilus cells and do show a chromaffin reaction. Berger<sup>7</sup> has reported the simultaneous presence of cells of both types in the newborn. Pheochrome nests about the ovary, however, are quite rare in the adult since in the human there is a general involution of chromaffin tissues shortly after birth.

Cell nests identical with the ovarian hilus cells have long been known to exist in the male gonad outside of the testis proper. Verocay,<sup>24</sup> in 1915, first studied these, and they have since been noted by numerous authors.<sup>25,26</sup> They may be found in the tunica albuginea, the hilus of the testis, the region of the rete testis, along the spermatic cord, and elsewhere. They have, furthermore, precisely the same relationship to nonmyelinated nerves as do ovarian hilus cells, and in fact are histologically indistinguishable from them. Berger<sup>5,8</sup> has studied these extratesticular Leydig cells extensively, as have Wieser,<sup>13</sup> Kohn,<sup>11</sup> Nelson,<sup>27</sup> and others.

Kohn<sup>11</sup> studied ovarian hilus cells in the newborn, and noted their prominence in an anencephalic infant. He found them as late as the 77th year of life. Brannon,<sup>21</sup> who studied a large series of ovaries, found abundant hilus cells in ovaries of 4 pregnant women, all cases of toxemia.

#### MORPHOLOGY OF OVARIAN HILUS CELLS

Over 100 ovaries (singly or in pairs) taken from routine surgical and autopsy material were studied. Since the hilus cells cannot be distinguished grossly and since they are distributed in irregularly scattered clumps, they may be absent from some sections cut through the hilus of an ovary but present in others. However, using a limited number of sections (4 to 6, on an average) hilus cells were identified in approximately 80 per cent of the adult ovaries studied. In the ovaries of 12 adult women a more thorough search was made and hilus cells were found in all cases. It is altogether probable that some of these cells are present in all adult ovaries. In general more hilus cells were found per section when the ovarian hilus and mesovarium were sectioned longitudinally after fixation.

## DISTRIBUTION

There was considerable variation both in the number and distribution of the ovarian hilus cells. They were present in the hilus and mesovarium, being somewhat more numerous at the lateral and medial poles of the hilus, and particularly near the junction of the ovarian ligament and ovary. Cell nests were seen frequently in the areolar tissue of the hilus close to its well defined junction with the ovarian stroma. However, nests were seen often much deeper in the mesovarium. Cell nests might be found close to the rete ovarii although no constant anatomic relationship was apparent. Occasional nests were incorporated in the ovarian stroma, but these were almost always close to the hilus and appeared to represent extensions of the hilus into the ovary proper.

The cells occurred in aggregates that varied considerably in size, shape, and number of cells. Irregularly shaped cell clusters often partially surrounded vessels and nerves in the vicinity. Unlike the occasional adrenal rests of the region, they never were encapsulated or delimited sharply from the surrounding tissues, but rather seemed indigenous to the hilar stroma.

## CYTOLOGY

The remarkable range of morphologic variations in the testicular Leydig cells is well known to all who have studied them. They vary from cells indistinguishable from the fibroblast, through elongate, polygonal, and oval forms containing an inconstant variety of intracytoplasmic inclusions, to rare giant multinucleated types. This protean variation in form might appear to offer difficulties in establishing the morphologic identity of hilus and Leydig cells. Actually, the reverse is the case, since the ovarian hilus cells reveal precisely the same types of morphologic variation.

The majority of ovarian hilus cells measured from 14 to 25  $\mu$  in diameter, although occasional larger forms were seen. Frequently each cell in a group was separated from its neighbor by a delicate collagenous fibril, as seen often with testicular Leydig cells. The cells for the most part were polygonal or oval although occasional elongate forms were seen.

The nucleus was vesicular and more constant morphologically than the cytoplasm. It was spherical, averaging 7 to 10  $\mu$  in diameter; occasionally it was ovoid or reniform. The chromatin clumps were coarse and rather sparse. Most cells contained one or two basophilic oval nucleoli. Occasionally three nucleoli were seen.

The cytoplasm was acidophilic, with a well marked granularity. A

narrow clear zone often was seen about the nucleus. Many cells were vacuolated but the degree of vacuolization was highly variable. Some cells had a zone of acidophilic granulation about the nucleus and vacuoles in the peripheral cytoplasm, an arrangement common in testicular Leydig cells. In one area the cells might be of smaller average size with a deep acidophilic and nonvacuolated cytoplasm and a darker nucleus, while adjacent areas might show larger cells with paler, more abundant cytoplasm and a clearer nucleus. The latter cells were more likely to show lipid vacuolization. These cell types may represent variations in the state of secretion of the cells.

Nelson<sup>27</sup> found giant multinucleated interstitial cells in 85 of 721 testes studied. Identical forms were found occasionally among the hilus cells. A group of these giant multinucleated ovarian hilus cells is illustrated in Figure 20.

### *Cytoplasmic Inclusions*

*Lipids.* The cytoplasmic vacuoles contained lipids which stained brilliantly with the usual fat stains such as sudan III. As with testicular Leydig cells, the lipid content varied considerably from one cell to another. No stainable lipid was present in some cells; others might show only fine dust-like, sudanophilic droplets; and in some the cytoplasm was filled with large, irregular masses of sudanophilic material. Some, but not all, of the lipid droplets were anisotropic.

*Pigment.* Small round granules of golden-brown pigment were present in the cytoplasm of some cells, in both stained and unstained sections. The pigment granules took up sudan III to some extent. This pigment probably belongs to the group of lipochromes, and is identical in appearance with the lipochrome of Leydig cells. As in the testis, it is more prominent in older patients.

*Crystalloids of Reinke.* Testicular Leydig cells in human beings may contain characteristic "crystalloidal" cytoplasmic inclusions. These curious structures were first described by Reinke<sup>28</sup> in 1896 and, although nothing has been learned of their function, they are generally accepted as structures peculiar to Leydig cells. However, they are found also in the ovarian hilus cells, a fact which greatly strengthens the morphologic evidence of identity.

The crystalloids of Reinke are acidophilic rod-like structures within the cytoplasm. They are often as long as the greatest diameter of the cell, at times measuring 30  $\mu$ , more commonly averaging 10 to 20  $\mu$ . The ends are rounded or rectangular with blunted corners. Often a straight, clear line bisects the structure longitudinally. Commonly a clear zone or halo surrounds the structure. They are frequently multiple in

a given cell, tending to a parallel arrangement. Smaller, narrower types are found which have tapering or pointed rather than blunt ends. At times the crystalloids appear to be extruded partially from the cytoplasm of the cell.

The crystalloids were found in only a small percentage of hilus cells (as is the case with testicular Leydig cells) and patient search was necessary. In many ovaries, even with the study of a great many sections, they were not found. When present, they usually had a patchy distribution, abundant in localized areas and absent in adjacent clusters of hilus cells. When a single crystalloid was seen, others generally were found in neighboring cells. The significance of this distribution is not understood. Crystalloids in abundance and with the same patchy distribution were found in both of the cases of masculinizing tumors to be described.

What little histochemical data are available suggest that the crystalloids are protein bodies. They take the acid dyes strongly, are not sudanophilic, not doubly refractile, nor do they dissolve in the fat solvents. Eberth<sup>20</sup> stated that they swell in 10 per cent potassium hydroxide and dissolve in hydrochloric acid with pepsin, although they are insoluble in 10 per cent hydrochloric acid, nitric acid, and acetic acid.

I have observed spherical acidophilic bodies of varying size in the cytoplasm of cells in close proximity with crystalloid-containing cells. These "hyaline" spheres had the same staining reactions as the crystalloids, and may represent precursors of them. They were particularly prominent in the tumor cells in case 2 where crystalloids also were very abundant. In some fields the majority of cells which did not contain crystalloids contained these bodies. Often they occupied most of the cytoplasm of the cell, at times compressing the nucleus to one pole. Structures that appeared to be transitional between the spherical bodies and the crystalloids were present. These intermediate forms were ellipsoids, or broad rectangles with rounded corners. Some are illustrated in the camera lucida drawing (Fig. 23). These observations suggest that the typical crystalloids may develop by gradual transformation from pre-existing spherical cytoplasmic inclusions.

#### RELATIONSHIP TO NERVES

There was a constant relationship of ovarian hilus cells to the non-myelinated nerves of the hilus. The cells were commonly found in masses irregularly ensheathing nerves. Even more striking was the presence of hilus cells, scattered singly or in groups, within a nerve trunk, lying between individual nonmyelinated nerve fibers and often producing localized bulging of the contour of the nerve. When nests were found

apparently isolated from nerves, serial sections almost always established contiguity with a nerve. I have confirmed the observations of Berger<sup>5,8</sup> and others that Leydig cells, located outside of the testis proper, have precisely the same relationship to nerve. In one instance, I also have observed a nonmyelinated nerve bundle within the testis proper, in which Leydig cells were dispersed in similar fashion within the nerve, separating adjacent nerve fibers.

#### RELATIONSHIP TO VESSELS

The ovarian hilus cells and their associated nerves often bear an intimate relationship to the vascular spaces of the ovarian hilus and mesovarium. The normal ovarian hilus has a remarkably complex vascular structure which has never been adequately explained. The bulk of the hilar tissue is made up of a tangled meshwork of small, tortuous, muscular arteries, large venous sinuses, and lymphatic spaces. Its vascular structure has recently been emphasized by the excellent injection preparations of Reynolds.<sup>30</sup> The number, size, and concentration of vessels seem far in excess of any reasonable vascular needs of so small an organ as the ovary.

Masses of hilus cells and nerve containing them were found in close association with large venous and lymphatic sinusoids. In fact, nerves containing or surrounded by hilus cells often formed distinct nodular protrusions into the lumina of vessels. In such areas the lumen appeared to be separated from the perineurium only by a layer of endothelial cells. Such structures are illustrated in Figures 7 and 8.

#### HYPERPLASIA OF HILUS CELLS ASSOCIATED WITH MASCULINIZATION

Two instances of hyperplasia of ovarian hilus cells associated with clinical masculinization may be mentioned briefly. Both cases will be reported in greater detail in a subsequent study.

The first of these, Mrs. R. M., a 41-year-old para II, gravida II, had shown masculinization for 8 years. Among the pertinent findings were a heavy beard requiring daily shaving, a deep voice, large clitoris, and a masculine, although somewhat obese, habitus. Menses occurred about every 2 months.

The second case, Mrs. V. P., a 50-year-old para I, gravida II, gave a history of masculinization of 3 years' duration. Previously a well developed female, she grew a heavy beard, and her voice deepened. Menstrual periods, which had been regular, ceased 5 months before admission. Pertinent findings included obesity of abdomen, thighs, and hips, hirsutism of face and body, an enlarged clitoris, and atrophic breasts.

Both patients were subjected to abdominal exploration and panhysterectomy. The adrenals in both patients were normal grossly at operation.

Tissue taken for biopsy of an adrenal in the first case was not remarkable. In both patients the ovaries were eight to ten times the normal



size for the age, and were fairly firm. Except for a single small follicular cyst in the first case, no cysts or follicles were present. Microscopic examination revealed field after field of ovarian stroma with only several corpora albicantia interspersed. Of greatest interest, however, was the presence in both cases of a significant increase in the number of ovarian hilus cells.

Rare instances of greatly enlarged ovaries associated with masculinization are described in the literature.<sup>31-31</sup> No very satisfactory explanation of the masculinization in such cases has been proposed, although "hyperthecosis"<sup>33,34</sup> of the ovaries, and pituitary stimulation<sup>32</sup> have been advanced. It seems likely that the hyperplasia of ovarian hilus cells, in the 2 cases cited, was largely responsible for the development of masculinization. Whether this increase of hilus cells was secondary to other hormonal stimulation cannot very well be discussed within the scope of this paper. The significance of the great increase in bulk of the ovarian stroma is obscure. In this regard, however, the relatively large size of the ovaries in the case with bilateral hilus cell tumors, presented below, is worth emphasizing.

#### HILUS CELL TUMORS WITH MASCULINIZATION

Berger,<sup>8</sup> in 1942, reported a case of masculinization associated with a small tumor of ovarian hilus cells. The patient, 50 years of age, had been masculinized for 18 years. There was facial hypertrichosis requiring shaving two or three times weekly, a masculine body build, deep voice with a prominent larynx, atrophy of the breasts, and an enlarged clitoris. In spite of these secondary sexual changes, she menstruated regularly. Following removal of ovaries, uterus, and tubes there was a significant regression of her masculinization. In the medial portion of one mesovarium there was a small tumor measuring 4.5 by 3.5 mm. composed of ovarian hilus cells. This is the only definite account of such a tumor which was found in the literature.

Another possible example of a similar neoplasm reported by Dreyfus and Barrozo do Amaral<sup>34a</sup> occurred in a female pseudo-hermaphrodite, but the picture was complicated by the simultaneous presence of aberrant corticoadrenal tumors in the region of the adrenal gland.

The following 2 examples of ovarian hilus cell tumors associated with clinical masculinization bring the total number of established cases to 3.\* It is likely that others exist but have not been accurately diagnosed.

\* Since this paper was submitted for publication, I have observed one additional case of hilus cell tumor, as well as 2 instances of hilus cell hyperplasia, all 3 associated with masculinizing syndromes.

*Case 1*

*Clinical History.* Mrs. C. G., a masculinized colored female, 86 years of age, was admitted to the Tulane Service of Charity Hospital on April 21, 1947, with a complaint of vaginal discharge and vague abdominal pain present for several months. Her childhood had not been remarkable and female characteristics had developed normally. She had borne three full-term children, all of whom had died in early infancy. Evidence of masculinization, as well as could be determined, dated from her 54th year, when she first began to grow a beard. Some time later her voice deepened. Because of her difficulty in recalling past events, little is known of her menstrual history, save that she had not menstruated nor had any vaginal bleeding for many years. No members of her family showed evidence of masculinization.

*Physical Examination.* Her features and habitus simulated those of an elderly male to a remarkable degree. She looked distinctly younger than her stated age and her skeletal musculature was remarkably well developed. She weighed 110 lbs.; her height was 136 cm. Her blood pressure varied between 140/88 and 164/100 mm. of Hg; pulse, between 58 and 70. She had a heavy mustache and a coarse, straggly beard. Her features were coarse and there was recession of the hairline at the forehead. The thyroid was not palpably enlarged. The thyroid cartilage was prominent and her voice deep. The breasts were small and atrophic and the nipples flat. A hard, nodular, nontender, movable mass was palpable in the lower abdomen, apparently arising within the pelvis and extending several centimeters above the symphysis. A male distribution of pubic hair was present; the labia majora and minora were atrophic. The clitoris was greatly enlarged, measuring 3.5 cm. in length by 1.5 cm. in diameter. The vaginal mucosa was not as atrophic as one might have predicted from the vulvar atrophy. The cervix was small and clean. Three or four stony masses projected from the sides of the uterus. The adnexae were not clearly outlined but it was felt that both ovaries were slightly enlarged and firm.

*Laboratory Studies.* Routine examinations of blood and urine were normal. Urinary concentration was good. The glucose tolerance test revealed a fasting level of 100 mg.;  $\frac{1}{2}$  hour, 135 mg.; 1 hour, 143 mg.; 2 hours, 103 mg.; 3 hours, 100 mg., per 100 cc. of blood. Twenty-four hour urinary 17-ketosteroids, as determined on three occasions by Dr. Albert Segaloff, were 6 mg., 9 mg., and 6 mg. The basal metabolic rate was -16.

Roentgenograms of the skull were normal, but those of the abdomen revealed multiple large areas of calcification in the lower abdomen and pelvis, compatible with calcified leiomyomas. Intravenous pyelograms were not remarkable.

The patient was considered a good surgical risk despite her age, and on May 1, 1947, was operated upon on the Gynecology Service. The adrenals were palpated at operation and were not enlarged. The ovaries, although of normal contour, were moderately enlarged for a woman of 86 years and the uterus was distorted by several partially calcified leiomyomas. A total hysterectomy and bilateral salpingo-oophorectomy were done. The postoperative course was uneventful save for a wound infection and she was discharged on June 20, 1947, in excellent health.

*Pathologic Examination*

The specimen consisted of an enlarged uterus with both tubes and ovaries attached. The cervical canal was stenotic for 2 mm. in the region of the external os. The uterus was grossly distorted by numerous leiomyomas, many of which were calcified. The endometrial cavity measured 6.5 cm. in length and 4 cm. in its greatest width. The endometrium was

pale pink, measuring less than 1 mm. in thickness. The tubes were not remarkable.

The ovaries were distinctly larger than would be expected from the advanced age of the patient. They corresponded roughly to ovaries of the 45 to 50 year age group, each measuring 3.5 by 2.5 by 2 cm. The serosal surfaces of both ovaries showed the usual pitting and corrugations. The ovarian tissue was firm but retained a slightly fleshy consistency. It was pinkish gray on section, with an indistinct tannish mottling. There were several corpora albicantia but no cystic structures present.

At the uterine pole of each ovarian hilus, lying within the mesovarium but contiguous with the ovarian stroma adjacent to the ovarian ligament, was a small, clearly demarcated tumor mass. The two tumors were remarkably symmetric in both position and size. They varied from a dark orange-brown to a dark olive-brown, and were of homogeneous fleshy consistency. The tumor at the left hilus measured 1 by 0.7 by 0.5 cm.; the tumor at the right hilus measured 1.2 by 0.8 by 0.5 cm.

*Histologic Examination.* The endometrium showed senile atrophy with cystic dilatation of the endometrial glands. The myometrium was atrophic. The leiomyomata showed considerable hyaline degeneration with extensive zones of calcification in some. There was chronic endocervicitis and dilatation of the endocervical glands. The tubes were not remarkable save for senile atrophic changes.

The ovaries did not show the advanced senile atrophy of the stroma expected in a woman of 86 years. The ovarian stroma was moderately cellular, comparable to that in the age group of 45 to 50 years. The tunica albuginea was of average thickness. A number of corpora albicantia were present. There were no follicular, thecal, or lutein cellular elements. Along the entire length of the ovarian hilus and adjacent mesovarium in both ovaries there was a striking abundance of hilus cell nests. The hilus cells showed the usual morphologic variations but were greatly increased in number. The usual relationship of hilus cell nests to nerves and vascular spaces was observed. Occasional crystalloids of Reinke were present within the cytoplasm of hilus cells. Figure 14 is a diagrammatic representation of the left ovary and adnexa as seen from behind.

*Histology of Tumors.* The bilateral tumors were composed exclusively of ovarian hilus cells, with a sparse accompanying stroma. They were not encapsulated. Occasional nonmyelinated nerve bundles lay adjacent to the periphery of the tumors and in some instances partially penetrated them. Neighboring hilus cells extended along the nerves so

that it was difficult in some regions to differentiate tumor cells from the hilus cells of the vicinity.

The two tumors were composed of well differentiated cells, many of which were indistinguishable from normal hilus cells. A considerable number, however, were larger, many measuring two and three times the diameter of average hilus cells. There was a greater variation in size and form in the tumor cells than in normal hilus cells. Many of the nuclei were irregularly shaped, but were otherwise comparable to the nuclei of hilus cells. Particularly toward the periphery of the tumors, the cells assumed elongate forms. A variable quantity of collagenous stroma was present in different parts of the tumor. Small groups of cells and often individual cells were surrounded by delicate collagenous fibrils. The cytoplasmic structures and staining reactions were identical with those of normal hilus cells.

Crystalloids of Reinke were abundant in the tumor cells, but the distribution was patchy, some areas containing numerous crystalloids, others being free of them. The crystalloids were mostly of the large variety, although occasional cells contained aggregates of small crystalloids. They were particularly well stained with the Masson trichrome stain and with phosphotungstic acid hematoxylin.

The cytoplasm was distinctly granular and acidophilic. Many cells showed marked granularity about the nucleus with vacuolization of the peripheral cytoplasm. With acid fuchsin, somewhat larger granules, 1 to 2  $\mu$  in diameter and lying within vacuoles, stained brightly. These were more prominent in the periphery of the cytoplasm. They correspond to the granules described by Whitehead<sup>35</sup> in his study of Leydig cells.

Mitochondria in the tumor cells were stained with phosphotungstic acid hematoxylin after preliminary mordanting of formalin-fixed material in 5 per cent aqueous ferric chloride after the method of Mallory. The mitochondria stained a deep blue. They were abundant in the form of round granules and short rods, duplicating the mitochondrial stains illustrated by von Winiwarter<sup>36</sup> in testicular Leydig cells. Some cells showed larger irregular clumps, small ring forms, and irregular networks in the peripheral cytoplasm. These probably represented mitochondria distorted by fixation as suggested by Duesberg<sup>37</sup> for the Leydig cells. They corresponded very closely to Duesberg's illustrations.

Many of the cells contained yellow-brown lipochrome pigment in small round granules in the cytoplasm, which probably accounted for the brownish color of the tumors. Sudan III stains of frozen sections revealed abundant but irregularly distributed lipid. Some cells were

free of sudanophilic material; the more vacuolated cells contained large amounts in the cytoplasm. Other cells contained only sparse small droplets of sudanophilic material. The lipochrome pigment took the stain to some extent. The variation in lipid content was comparable to that in Leydig cells. Some of the stainable lipid was doubly refractile when examined with the polarizing microscope.

### Case 2

*Clinical History.* Mrs. E. W., a white woman, 64 years old, was admitted to the Tulane Medical Service of Charity Hospital on April 18, 1947. During the previous 2 years she had developed a beard and mustache, as well as increased hair on the arms and legs. Beard growth averaged  $\frac{1}{4}$  inch weekly. During this period her voice had deepened. Two years previously she had been a normal female. She had borne two normal full-term children, now 35 and 33 years old. Her family history was not contributory. Her menstrual periods, which had been regular, ceased at the age of 40. There had been no subsequent vaginal bleeding except for an episode of spotting 8 months before admission.

*Physical Examination.* The patient was short, moderately obese, and weighed 146 lbs. There was congenital absence of the left arm below the elbow. The important findings included a marked facial hirsutism, and less marked hirsutism of the arms and legs. There was some recession of the hairline at the forehead. The pubic hair showed a tendency toward masculine distribution. The features were heavy and appeared masculine. The thyroid cartilage was slightly enlarged. Breasts and abdomen were obese. The blood pressure on different occasions varied between 110/60 and 146/96 mm. of Hg. On pelvic examination the clitoris was not grossly enlarged. A senile vaginitis was present, and there was a second degree cystocele. The uterus and adnexae were not definitely outlined.

*Laboratory Findings.* Results of routine examinations of blood and urine were within normal limits. A glucose tolerance test showed a fasting blood sugar of 103 mg.;  $\frac{1}{2}$  hour, 167 mg.; 1 hour, 121 mg.; 2 hours, 103 mg.; 3 hours, 105 mg., per 100 cc. of blood. Basal metabolic rates on three occasions were +16, +16, and +25. The Kline and Kolmer tests were negative. Roentgenograms of the skull and chest and retrograde pyelograms were not contributory. Determination of the 24-hour urinary 17-ketosteroids by Dr. Albert Segaloff showed 7.5 mg.

The patient was transferred to the Tulane Gynecology Service and a bilateral salpingo-oophorectomy and supracervical hysterectomy were performed on May 22, 1947. At operation the adrenals were not palpably enlarged. Her postoperative course was uneventful.

When seen 2 months after operation she complained of occasional hot flashes. Eight months after operation her voice had resumed a normal female pitch and there was a definite reduction in facial hirsutism.

### Pathologic Examination

The uterus, removed supracervically, was of average size and contained no leiomyomas. The uterine cavity was small, with a thin endometrium. The tubes, except for slight thickening of the walls, were not remarkable.

The left ovary measured 2.8 by 1.3 by 1 cm.; the right ovary, 2.5 by 1.5 by 1.5 cm. Both were firm and grayish with the usual surface cor-

rugations. On section the ovarian stroma was grayish tan and free of cysts. In the inner medullary portion of the right ovary, midway between the poles, there was a spherical, yellow tumor nodule measuring 1 cm. in diameter. It was unencapsulated and fleshy. The tumor was surrounded by ovarian stroma for the most part, except for a portion which extended into the hilus region and adjacent mesovarium.

*Histologic Examination.* The endometrium was atrophic, with cystic dilatation of the endometrial glands. Moderate adenomyosis was present. The tubes showed healed chronic salpingitis.

The ovaries contained multiple corpora albicantia. The ovarian stroma showed moderate fibrosis and atrophy compatible with the age of the patient. The hilus cells in both right and left mesovaria were moderately increased in number although there was less hyperplasia than in case 1. Crystalloids of Reinke were fairly numerous. Nests of hilus cells lay in close proximity to the portion of tumor within the mesovarium. However, these were separated from the tumor, which was sharply circumscribed although not encapsulated.

*Histology of Tumor.* Sections of the tumor were prepared with hematoxylin and eosin, Masson trichrome, and sudan III stains. The histologic findings were very similar to those of case 1. The tumor was composed of well differentiated hilus cells, polyhedral and somewhat larger than average hilus cells. Mitotic figures were seen rarely. Few cells of the elongated form noted in case 1 were present, and the stroma was sparse and delicate. Nuclear and cytoplasmic details showed the same variations. Lipochrome pigment was present but less abundant. Stainable lipid was similar in amount and distribution to that in the tumors of case 1. Some of the lipid was doubly refractile.

Crystalloids of Reinke were present in even greater abundance than in case 1. Most of these were of the large type, some measuring as much as 35  $\mu$  in length. Occasional aggregates of smaller crystalloids were present also. As previously noted, numerous cells containing round acidophilic bodies with the same staining reactions as crystalloids were present. These may represent precursors of crystalloids. They were noted also in the hilus cells outside of the tumor and were more abundant than in case 1.

#### EVIDENCE OF FUNCTIONAL ACTIVITY OF OVARIAN HILUS CELLS

In these 4 patients with masculinization and with tumors or hyperplasia of hilus cells, the evidence is reasonably good that the hilus cells were functionally important. Do the ovarian hilus cells in the normal adult female produce androgens or do they merely represent vestigial structures? A precise answer is not possible in the present

state of our knowledge. The following facts, however, suggest that the cells normally have a secretory function.

The ovarian hilus cells are present at birth and can be identified during the first year or so of life. Following this and until the age of puberty they are absent or at least difficult to find. They reappear at puberty and persist through adult life, tending to decline in old age.<sup>12</sup> They are particularly prominent during pregnancy and at the menopause. These chronologic relationships suggest functional activity.

Furthermore, the cells have the histologic appearance of actively secreting cells. Cytoplasmic granules and lipid-containing vacuoles are prominent as they are in testicular Leydig cells. The histologic variations in the cells, as in testicular Leydig cells, suggest a life cycle of secretory activity. Crystalloids of Reinke in testicular Leydig cells do not appear until the age of puberty, when the cells become active. The only instance that I have seen of crystalloids of Reinke in the testes of a child occurred in a 4-year-old boy with precocious puberty of so-called constitutional or idiopathic type. Spermatogenesis was active and the Leydig cells were well developed, containing abundant crystalloids. Although the precise importance of the crystalloids is unknown, they appear to be associated (although inconstantly) with functional activity of the cells. The presence of crystalloids, then, in the hilus cells of normal adult women may reflect their secretory activity.

Finally, I should like to present evidence that the ovarian hilus cells respond to stimulation by chorionic gonadotropin. Ovaries from patients who had received 10,000 units of purified chorionic gonadotropin daily for at least 2 weeks were studied histologically.\* Injections were begun shortly after the estimated time of ovulation. At this dosage, as reported by Brown and Bradbury,<sup>38</sup> menstruation may be delayed for 7 or more days. Significant changes were noted in the ovarian hilus cells in the 3 cases studied thus far. In all, the hilus cells were abundant and showed a severe though patchy degeneration (Fig. 10). In such areas the cytoplasm was dense and deeply acidophilic, cell boundaries were lost so that adjacent cells appeared to fuse together, and the nuclei were distorted and pyknotic, with a tendency for adjacent nuclei to clump together. Hilus cells in other areas were normal in appearance. A similar degenerative change in hilus cells was noted in the opposite ovary in a case of teratoma in which urinary gonadotropin was elevated. Brannon<sup>21</sup> reported similar degenerative changes in the hilus cells of a woman dying of pernicious vomiting of pregnancy. The possi-

\* I am indebted to Drs. Brown and Bradbury for supplying me with ovarian tissue and adjacent mesovarium from one of their cases.

bility that the changes which he noted were due to chorionic gonadotropin stimulation must be considered.

Of particular interest was the presence of mitotic figures in the hilus cells of one case receiving large doses of chorionic gonadotropin. This case also showed degenerative changes in other groups of hilus cells. Mitotic figures are so rare in normal cells that none was found in over 100 routine specimens examined, although rare mitotic figures were present in the hilus cell tumors. Mitotic figures are equally rare in testicular Leydig cells.<sup>39</sup> The presence, then, of significant numbers of mitotic figures in the hilus cells of a patient who received chorionic gonadotropin is probably not fortuitous.

The evaluation of these morphologic changes in hilus cells with chorionic gonadotropin is not simple. However, as is well known, chorionic gonadotropin stimulates the growth and secretion of Leydig cells in the male. It is possible that the degenerative changes in the hilus cells are an exhaustion effect, particularly since the dosage of gonadotropin used was considerable. Nevertheless, the fact that hilus cells show responses to hormonal stimulation is additional evidence suggesting functional activity.

#### DISCUSSION

Specific tumors of ovarian hilus cells are rare, as are ovarian masculinizing tumors in general. The case reported by Berger<sup>8</sup> and the two here presented are in fact the only established cases in the literature. It seems altogether likely, however, that some hilus cell tumors have been misdiagnosed as arrhenoblastomas, adrenal rest tumors, or luteomas, especially since the specific diagnosis of the masculinizing ovarian tumors is frequently a source of controversy among pathologists. Critical study of several reports of atypical masculinizing ovarian tumors strongly suggests that some of them may represent hilus cell tumors.

Clearly the hilus cell tumors should be separated from the broad and rather loose category of arrhenoblastomas. In the hilus cell tumors one finds neither the differentiated tubular structures nor the undifferentiated sarcoma-like pattern of the arrhenoblastoma. Unlike the more complex arrhenoblastoma, the hilus cell tumor represents a benign, well differentiated neoplasm composed of cells of a single type, which is similar to that of cells present in the normal ovarian hilus.

Although it is possible that tumors of hilus cells could be confused with adrenal rest tumors, there should be little difficulty in distinguishing adrenal rests of the ovarian hilus from hilus cell nests. The adrenal rests are usually yellow, spherical nodules, 1 to 3 mm. in diameter, sharply circumscribed and encapsulated. The hilus cells, in contrast, are



never encapsulated and tend to be dispersed in many small, irregular nests within the connective tissue of the hilus. Furthermore, the adrenal rests have an organoid structure, with cell cords centripetally arranged and duplicating normal adrenal cortex. Usually an indication of adrenal cortical zones is recognizable. The cells of the zona fasciculata are crowded with uniformly dispersed lipid-containing vacuoles, unlike the irregular and sparser lipid vacuoles of hilus cells. During the past year, we have seen in our laboratory at least six adrenal rests located in tissues adjacent to the adult ovary, as well as comparable structures adjacent to the testes in newborns. They are not likely to be confused with hilus cell nests. A typical example of an adrenal rest in an ovarian hilus is illustrated in Figure 25.

Certain aspects of the three known instances of masculinizing hilus cell tumors are worthy of comment, although generalizations are impossible on so small a group. In all, the tumors were of small size, 1 cm. or less in diameter. They were composed exclusively of ovarian hilus cells, well differentiated and apparently entirely benign. Crystalloids of Reinke, which, when present, are the most convincing identifying feature of these cells, were abundant in the tumors reported here. They were not present in Berger's case, nor need they be considered as a constant finding since they are present in only a fraction of normal hilus cells.

Excretion of urinary 17-ketosteroids in the two cases reported here were within normal limits, as may also be the case in women with masculinization due to arrhenoblastoma.<sup>40,41</sup> In contrast, 17-ketosteroids are more commonly elevated in masculinization due to adrenal lesions.

The persistence of menses in Berger's case and the lack of enlargement of the clitoris in case 2 indicate that the masculinizing syndrome need not be complete. The absence of hypertrophy of the clitoris in the latter case may reflect a difference in end-organ response to hormonal stimulation. Women treated with fairly large doses of testosterone for metastatic breast carcinoma show a striking difference in clitoris response, ranging from lack of detectable growth to marked hypertrophy.<sup>42</sup>

It is now well established that the normal adult female produces androgens. There is, in fact, but little difference either qualitatively or quantitatively in the urinary excretion of 17-ketosteroids in males and females.<sup>43,44</sup> The source of androgens in the female is generally considered to be the adrenal cortex. There is, however, considerable experimental evidence that the ovary, too, is capable of elaborating male sex hormones.<sup>45-49</sup> The specific cell producing these ovarian androgens is not clearly established although some investigators implicate lutein cells or lutein-like cells. It seems likely that in the human, one source of

androgen is the ovarian hilus cell, although its quantitative importance (compared, let us say, to the adrenal) is difficult to evaluate. The morphologic identity of the hilus cells with Leydig cells, their cytologic characteristics as secreting cells, their response to chorionic gonadotropin, and the evidence of masculinization with either tumors or hyperplasia of these cells all point to androgen production.

Further histochemical and physiologic investigations are needed to clarify the rôle of these cells in the normal female. The relationship of the hilus cells to sympathetic nerves and vascular spaces seems too striking to be fortuitous. That these anatomic relations may reflect some special physiologic mechanisms is an intriguing possibility worthy of further investigation.

#### SUMMARY

Cells morphologically identical with testicular Leydig cells are regularly found in the human ovarian hilus and mesovarium. These ovarian hilus cells have a constant and intimate relationship to nonmyelinated nerves and vascular spaces. A similar relationship of Leydig cells to nerves and vessels is seen in the testicular hilus and adjacent structures.

Morphologic evidence, including the prominence of these cells at puberty, during pregnancy, and at the menopause, suggests functional activity. There is also evidence that they are responsive to stimulation by chorionic gonadotropin.

In the 2 cases of specific tumors of these cells and 2 cases of hyperplasia which are reported, there was masculinization.

The ovarian hilus cells may represent a further source of androgen in the normal female in addition to that derived from the adrenal cortex.

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[ Illustrations follow ]

## DESCRIPTION OF PLATES

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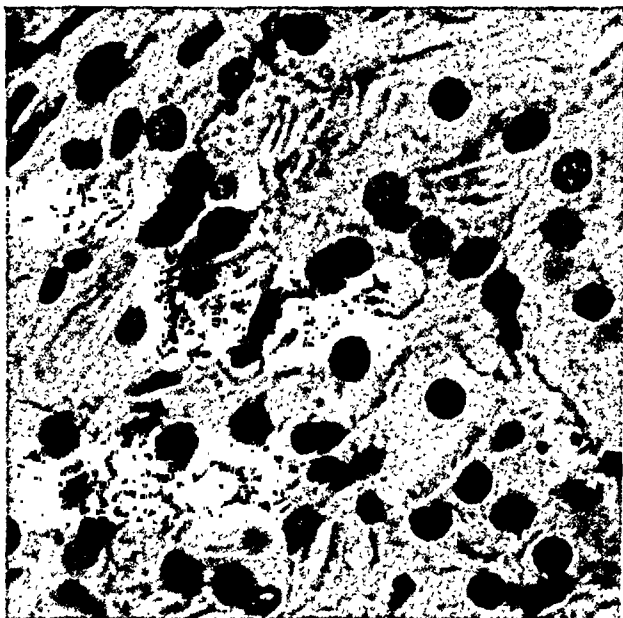
### PLATE 71

- FIG. 1. Two typical crystalloids of Reinke in the Leydig cells of the testis of a boy, 4 years old, with precocious puberty of idiopathic or constitutional type. Such crystalloids are a normal finding in mature testes. Crystalloids are seen in the lower left field; seminiferous tubules, above and lower right. (Slide was provided through the courtesy of Dr. Albert Segaloff.) Masson's trichrome stain.  $\times 500$ .
- FIG. 2. Ovarian hilus cells with abundant crystalloids of Reinke from a normal female, 39 years old, operated upon for uterine leiomyomas. Of note are the granular and vacuolated cytoplasm and acidophilic crystalloids. Hematoxylin and eosin stain.  $\times 580$ .
- FIG. 3. Ovarian hilus cells within and adjacent to a nonmyelinated nerve trunk of the mesovarium, a typical picture that may be seen in the hilus of normal ovaries. This is from the ovarian hilus at some distance from the hilus cell tumor in case 1. Hematoxylin and eosin stain.  $\times 170$ .
- FIG. 4. Hilus cell tumor in case 2, a 64-year-old masculinized female, showing large crystalloids of Reinke in tumor cells. Hematoxylin and eosin stain.  $\times 670$ .
- FIG. 5. Hilus cell tumor in case 1, an 86-year-old masculinized female, showing a variable distribution of sudanophilic substance in the cytoplasm of the cells. Frozen section: sudan III and hematoxylin stains.  $\times 500$ .
- FIG. 6. Hilus cell tumor in case 1, an 86-year-old masculinized female. Several crystalloids of Reinke are seen in one cell. The cells are larger than normal hilus cells, and the cytoplasm is abundant. A delicate collagenous fibril surrounds each cell. The brown pigment is lipochrome pigment. Masson's trichrome stain.  $\times 450$ .

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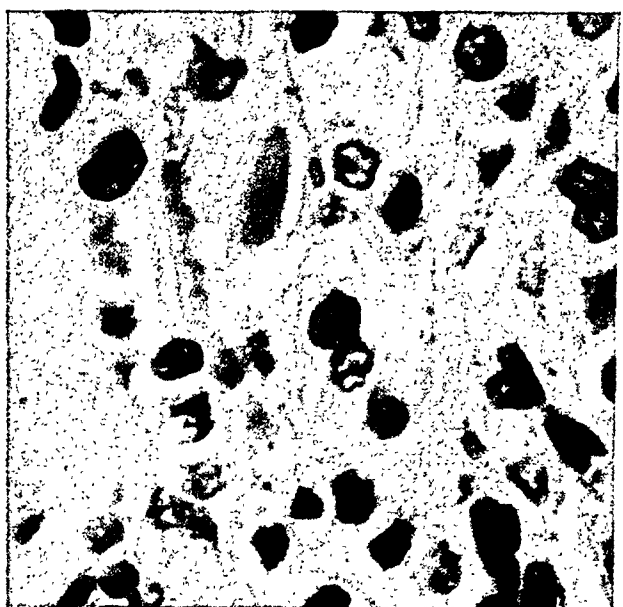
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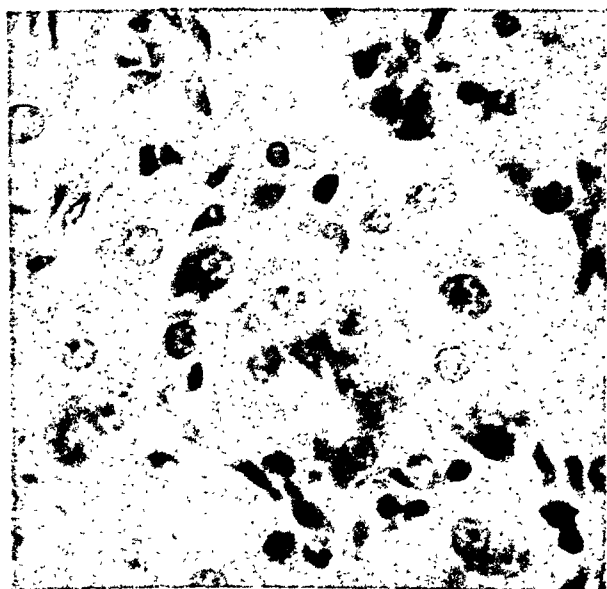
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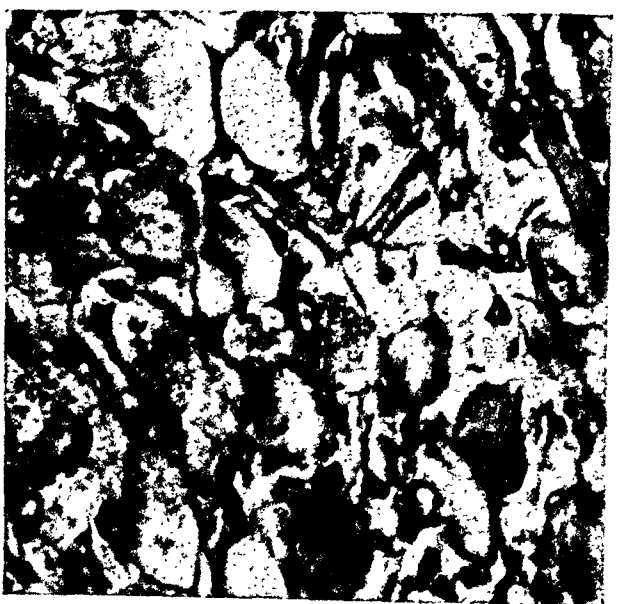
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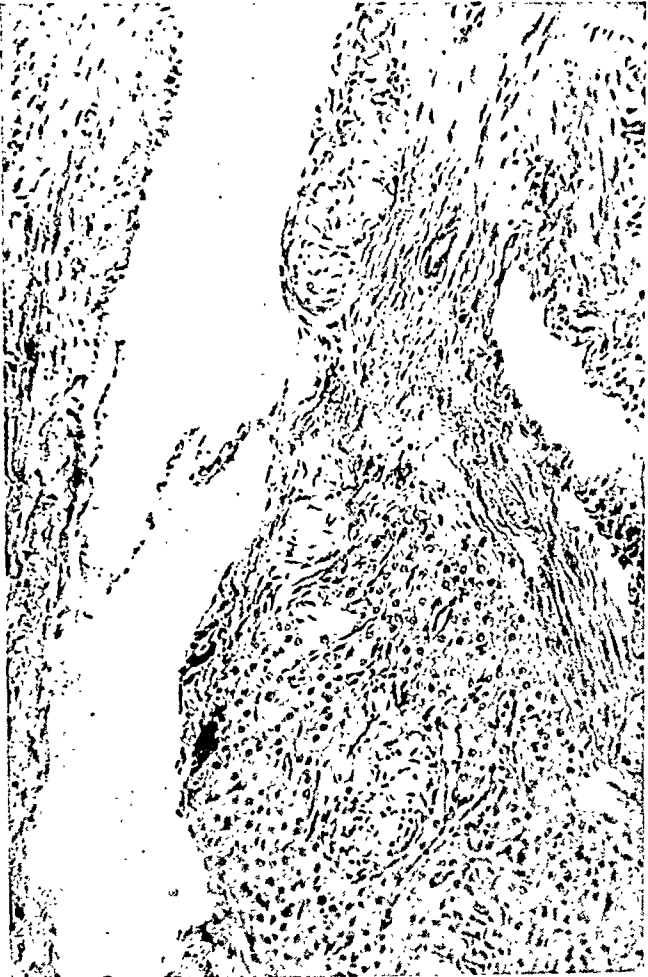
Sternberg

Ovarian Hilus Cells

PLATE 72

- FIG. 7. Nest of hilus cells and associated myelinated nerves protruding into a dilated lymphatic space in the mesovarium of a normal woman, 30 years old. Hematoxylin and eosin stain.  $\times 120$ .
- FIG. 8. Protrusion of nonmyelinated nerve and hilus cells into a dilated lymphatic space, from the same case as Figure 7. Hilus cells lie between nerve fibers. Only a layer of endothelium separates the structure from the lumen of the lymphatic. Hematoxylin and eosin stain.  $\times 250$ .
- FIG. 9. Hilus cells, with a mitotic figure in the lower part of the field, from a patient who received 10,000 I.U. of chorionic gonadotropin for 19 days. The nonmyelinated nerve bundle, upper right, contains hilus cells between nerve fibers. Hematoxylin and eosin stain.  $\times 490$ .
- FIG. 10. Hilus cells and nonmyelinated nerve in another portion of the mesovarium from the same patient as Figure 9. The hilus cells show degenerative changes, with dark, acidophilic, nonvacuolated cytoplasm, loss of cell borders, nuclear pyknosis, and clumping of nuclei. Masson's trichrome stain.  $\times 390$ .

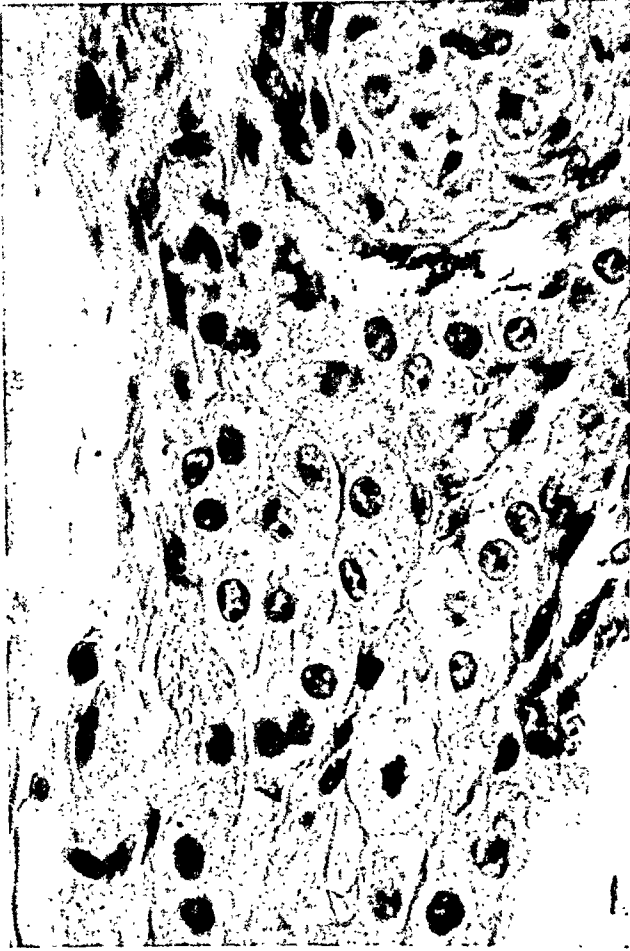
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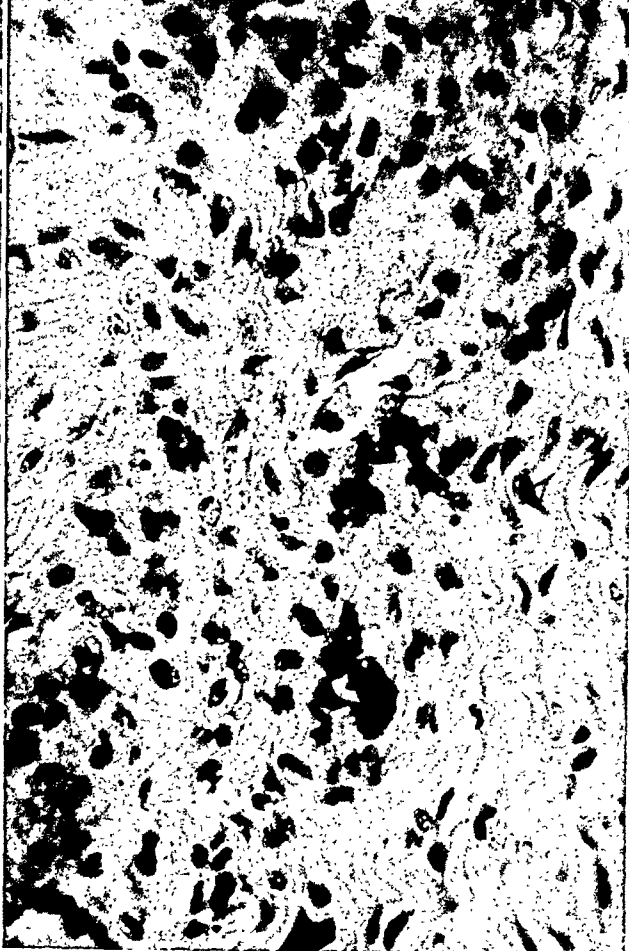




PLATE 73

FIG. 11. Photograph of patient, case 1, an 86-year-old colored masculinized female with bilateral ovarian hilus cell tumors. Of note are the masculine features, mustache, and beard growth. (Published with the written permission of the patient.)

FIG. 12. Photograph of enlarged clitoris, case 1.

FIG. 13. Photograph of ovaries with ovarian hilus tumors, case 1. The ovaries have been split longitudinally toward the hilus, and laid open before photographing. The dark areas in the upper portion of the left ovary and in the lower portion of the right ovary are the tumors. They were located symmetrically at the medial pole of each ovarian hilus.

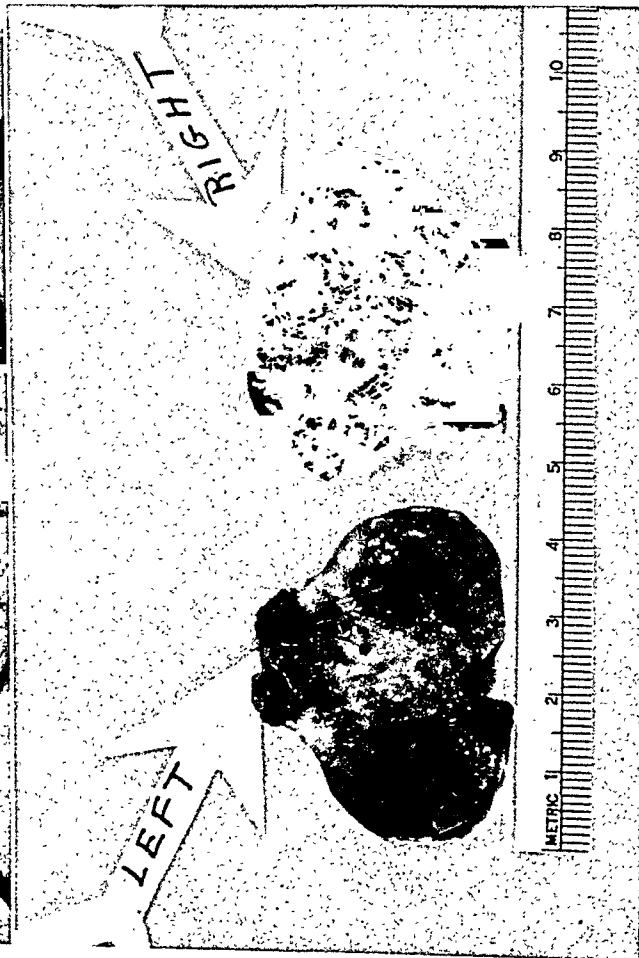
FIG. 14. Diagram of left ovary, case 1, showing relationship of the tumor, ovarian hilus, and adjacent structures. The area within the dotted line is plotted by projection from actual microscopic sections and represents the ovarian hilus and adjacent mesovarium. The large black area represents the hilus cell tumor. The smaller black islands represent hyperplastic nests of hilus cells. The ovarian ligament is to the right of the tumor.



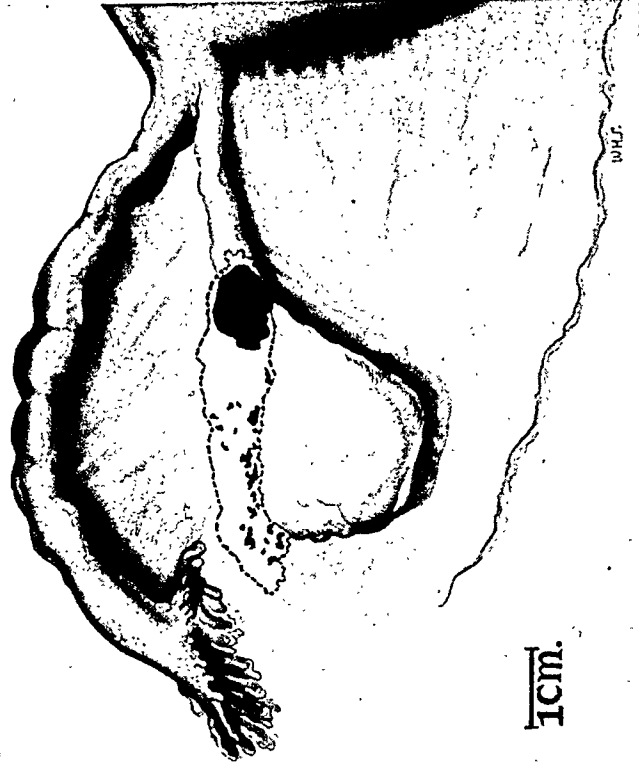
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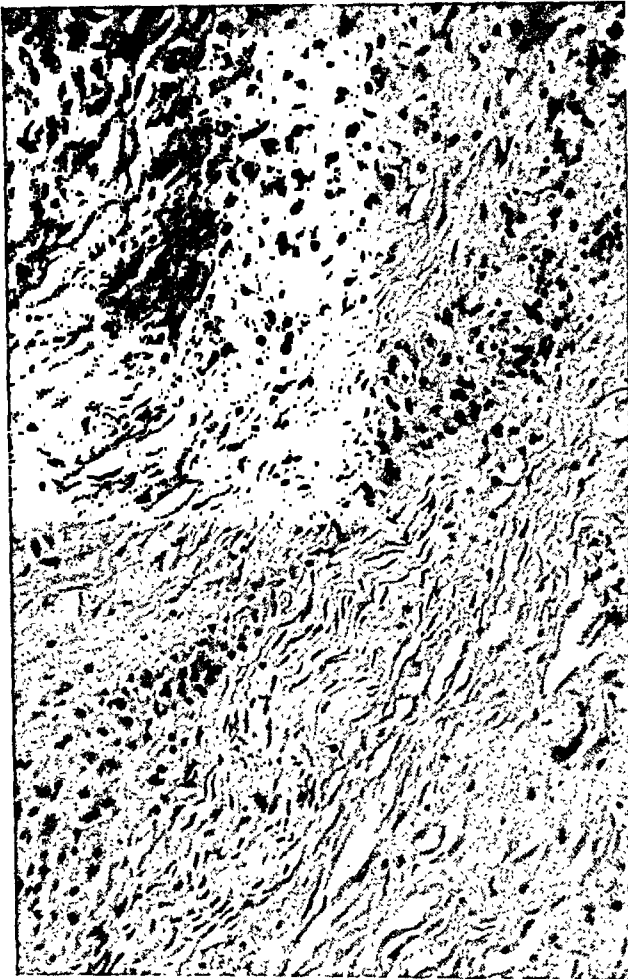


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PLATE 74

- FIG. 15. Ovarian hilus cells and nonmyelinated nerve at the periphery of the hilus cell tumor, case 1. Hematoxylin and eosin stain.  $\times 160$ .
- FIG. 16. Ovarian hilus cell tumor, case 1, showing elongated cell forms and greater morphologic variation than normal hilus cells. Crystalloid of Reinke is seen above and to the right of center. Two crystalloids are cut obliquely in the lower left field. Masson's trichrome stain.  $\times 500$ .
- FIG. 17. Crystalloid of Reinke from the hilus cell tumor, case 1. There is a clear space about the crystalloid. Phosphotungstic acid hematoxylin stain.  $\times 800$ .
- FIG. 18. Two crystalloids of Reinke from the hilus cell tumor, case 1. Delicate collagenous fibrils surround the cells. Phosphotungstic acid hematoxylin stain.  $\times 650$ .
- FIG. 19. Unusually large crystalloid of Reinke from the hilus cell tumor, case 1, with a surrounding clear space and longitudinal cleft. Phosphotungstic acid hematoxylin stain.  $\times 800$ .
- FIG. 20. Giant multinucleated hilus cells from the mesovarium of case 1, at some distance from the tumor. This is an occasional normal variation among hilus cells. Testicular Leydig cells may show similar forms. Hematoxylin and eosin stain.  $\times 480$ .

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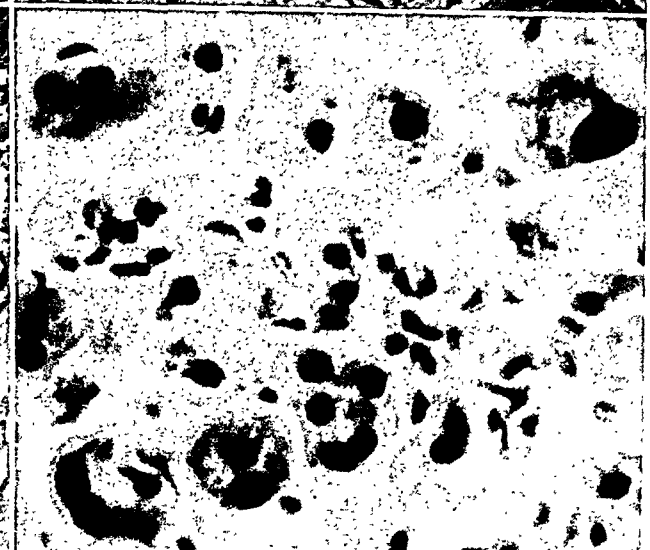
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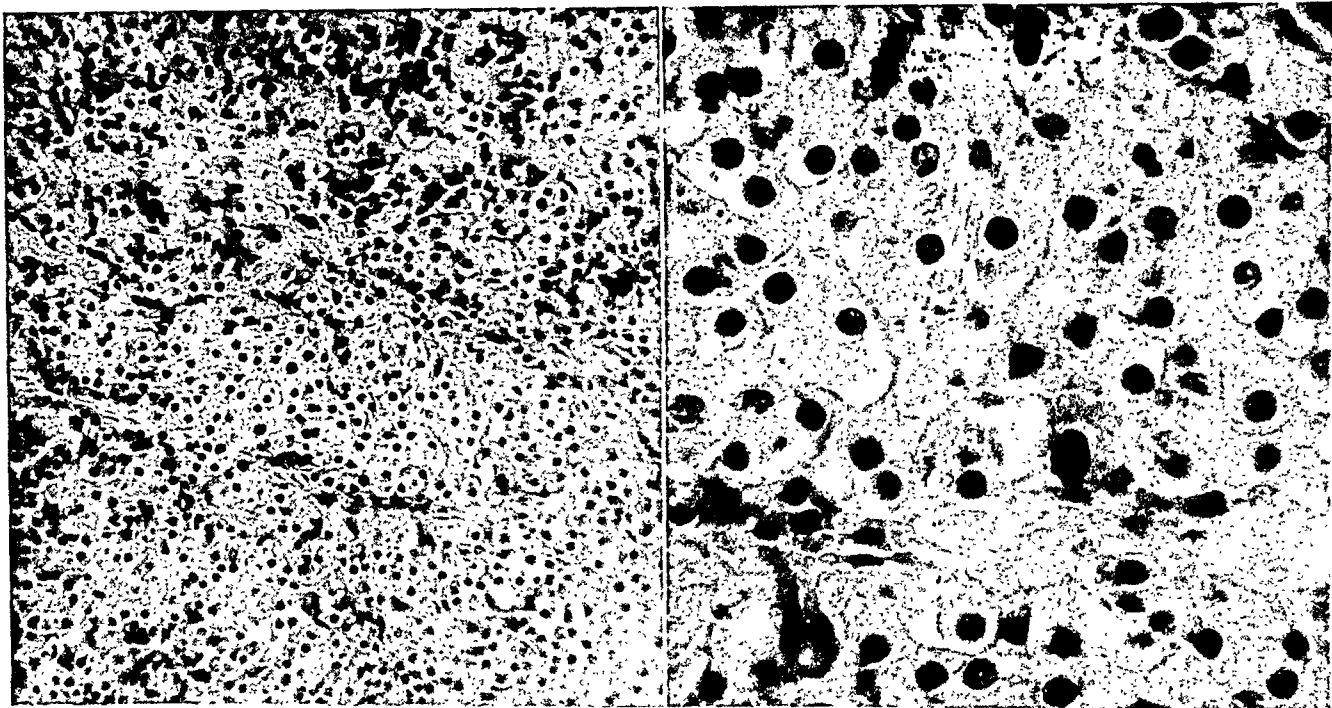
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## PLATE 75

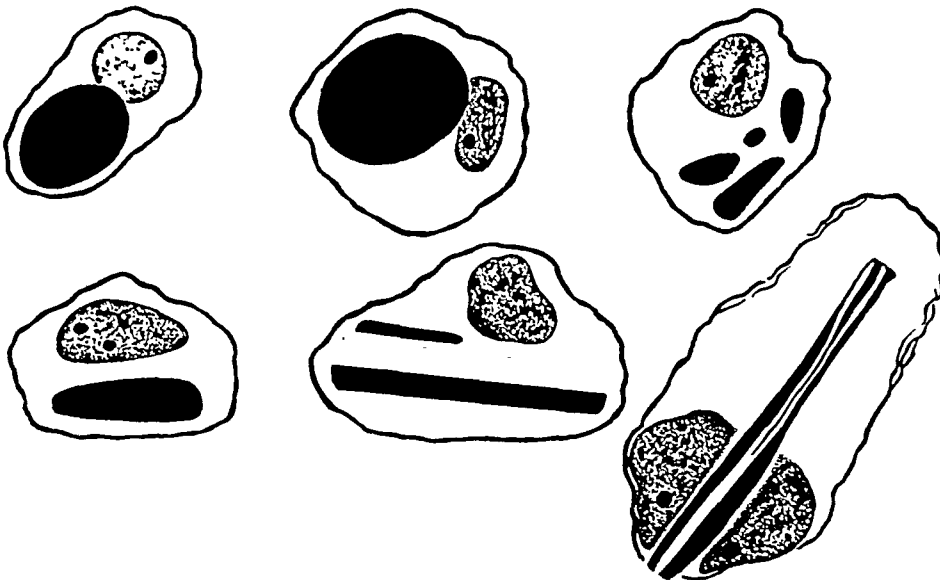
- FIG. 21. Ovarian hilus cell tumor from case 2, a masculinized white female, 64 years old. The cells are very similar to normal hilus cells. Hematoxylin and eosin stain.  $\times 160$ .
- FIG. 22. Ovarian hilus cell tumor from case 2. Of note is the granular cytoplasm. Hematoxylin and eosin stain.  $\times 670$ .
- FIG. 23. Camera lucida drawing of tumor cells of case 2, showing "transition" of intracytoplasmic hyaline spherical bodies to crystalloids. Cells 1 and 2 (counting from upper left) contain ovoid and spherical bodies; cells 3 and 4 contain "transition" forms; cells 5 and 6 contain well developed crystalloids of Reinke.
- FIG. 24. Photograph of masculinized 64-year-old white female, case 2, showing masculine features, recession of hairline, and mustache and beard growth. (Published with the written permission of the patient.)
- FIG. 25. Typical adrenal rest in an ovarian hilus, an incidental finding in a routine surgical specimen from a patient 41 years old. Of note are the capsule, zone formation, organoid structure and simulation of normal cortex. For comparison with hilus cell nests. Hematoxylin and eosin stain.  $\times 120$ .

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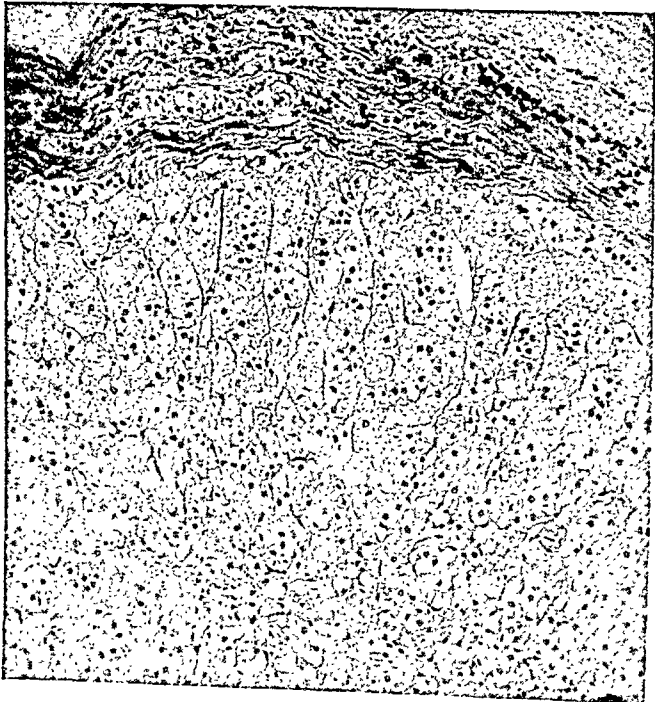
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# OXYPHILIC GRANULAR CELL ADENOMA OF THE PAROTID GLAND (ONCOCYTOMA)

## REPORT OF FIVE CASES AND STUDY OF OXYPHILIC GRANULAR CELLS (ONCOCYTES) IN NORMAL PAROTID GLANDS \*

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Although the parotid gland is the most common site of neoplasms of the salivary glands,<sup>1-5</sup> a pure adenoma in this location is considered a rarity.<sup>2-11</sup> McFarland<sup>9</sup> reported a "possible case," reviewed the literature, and concluded that "nearly all, if not, indeed, all, of the described adenomas are but mixed tumors of unusual appearance." This statement is now no longer valid since the literature contains several reports of true adenomas of which McFarland's case is a good example. A study of the reported adenomas leads to the impression that some of them belong to a definite neoplastic entity, because of the similarity of their cellular components. The characteristic cells have been variously designated. They are epithelial in origin and larger than the normal cells of the parotid gland; they have granular or reticulated oxyphilic cytoplasm, according to the stain employed. Their nuclei are dark-staining, frequently pyknotic, and with irregular contours.

Schaffer<sup>12</sup> was the first to describe these cells as "granular swollen cells." He found them in the ducts and acini of salivary glands of the tongue, uvula, pharynx, and esophagus. Pischinger<sup>13</sup> described them in the sublingual gland. Zimmermann<sup>14</sup> observed them in the trachea, floor of the mouth, and sublingual glands. Because of the characteristics of their nuclei he called them pyknocytes. Hamperl<sup>15</sup> studied the uvular, sublingual, and submaxillary glands of 85 persons whose ages ranged from infancy to 96 years. He gave a very complete and detailed description of the same cells and called them "onkocytes" because of their increased size. He found them occasionally in persons under the age of 50, but they occurred more frequently between 50 and 70, and were present practically always in those over 70 years of age. These peculiar cells sometimes formed tumor-like areas of hyperplasia disposed as solid or adenomatous foci.<sup>15,16</sup> In addition he described transitional forms between both ductal and acinar cells and the "onkocytes."<sup>15</sup> Steinhardt<sup>11</sup> examined the sublingual, submaxillary, and parotid glands of 65 persons and found 8 examples of what he thought were the "transitional form"

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between normal cells and the "onkocytes" described by Hamperl<sup>15</sup>; these "transitional forms" were found in 5 persons over 80 years of age and in 3 between 60 and 80 years. Steinhardt failed to mention specifically in which of the glands he found the cells. He also observed nodular hyperplasia in the submaxillary glands of 2 of his cases; one of these was of the solid and the other of the adenomatous papillary type. Hamperl<sup>17</sup> wrote that the cells of the "transitional form" of Steinhardt were "onkocytes." Škorpil<sup>10</sup> mentioned that in 100 salivary glands he found 5 examples of "oncocytes" forming nodular hyperplasia. The areas of hyperplasia were present in submaxillary, sublingual, and nasal mucosal glands. Stout<sup>18</sup> found cells of the same type in the bronchi of adults and discussed their possible relationship to the cells of bronchial adenomas. Nohteri<sup>19</sup> studied the mucous membrane of the nose and of the larynx of 41 and 37 persons, respectively, and his results were similar to those of Hamperl.<sup>15</sup> He found "oncocytes" only in persons over 52 years of age and regularly in those over 70. In addition, he described a cyst in the larynx composed of "oncocytes."

"Oncocytes" have been found also in other organs. Hamperl<sup>20</sup> listed the following: Parotid, submaxillary, sublingual, and minor salivary glands; thyroid gland, parathyroid gland, hypophysis (anterior and posterior lobes), testicle, fallopian tube, pancreas, liver, stomach, and the glands of the pharynx, trachea, and esophagus. Hamperl<sup>17</sup> also mentioned that Veratti<sup>21</sup> had noted similar cells in the salivary glands of dogs.

McFarland<sup>9</sup> reported what seems to be the first proved case of pure adenoma of the parotid gland composed of the oxyphilic granular cells.\* At that time he was not convinced that the lesion was a true adenoma, but seemed inclined to consider it as a mixed tumor of unusual appearance as shown by his conclusion: "The occurrence of gland-like tumors (adenomas) and of tumors containing varying quantities of glandular tissue is no indication that such tumors arise from the glandular tissue proper, but simply goes to show that glandular tissue may sometimes preponderate over other tissue components of mixed tumors." This view was restated in 1936<sup>22</sup> when, on commenting about the recurrence of the tumor after 12 years, he said: "Subsequent events fully justified its original classification as a mixed tumor."<sup>23</sup> However, in 1942<sup>24</sup> and 1943<sup>25</sup> he referred to this adenoma as a "Hürthle cell tumor (onchocytoma)" and placed it under the group of "tumors mistaken for mixed tumors."

\* The term "oxyphilic granular cell" will be used as a synonym of "oncocyte" and of other terms used to designate the cells under discussion.

Gruenfeld and Jorstad<sup>26</sup> reported the second example. They were the first to point out the similarity of the "onkocytes" of Hamperl to the cells of the case reported by McFarland as well as to those of their own. Their case appeared to be of multicentric origin. They expressed the opinion that it had originated from the duct system, and they called their lesion an "onkocyte tumor." Harris<sup>27</sup> reported a case which seemed to be of multicentric origin from the ducts. He objected to the name onkocytoma and stated: "Inasmuch as the onkocyte has also been named pyknocyte, this tumor could be called pyknocytoma, but the need for any such name is not apparent." Škorpil<sup>10</sup> reported 4 cases of adenoma of which the fourth was another example of parotid tumor composed of "onkocytes." As did the two previous authors, Škorpil stated that the tumor arose from the ducts, and added that the name "onkocitāres" adenoma or "onkocytoma" would be appropriate for such a tumor. Ackerman<sup>6</sup> recorded another example of adenoma and tabulated the reported cases up to that date. He reported his case under the name "oncocyoma of the parotid gland." Lloyd<sup>8</sup> reported the most recent case of parotid adenoma of the type under review, which is case 8 of his paper. He used the name "oncocyoma of the parotid" to designate his tumor. The only other proved case of tumor of this type found in the salivary glands is that of Ahlbom<sup>1</sup> who reported an adenoma of the hard palate which probably arose from the minor salivary glands of that region.

Besides these cases which can definitely be accepted as examples of the type of tumor composed of oxyphilic granular cells, there are some others which may be considered as possibly belonging to the same group, and also a few which have been included by some authors without sufficient justification. One probable case is the first of the two adenomas reported by Hüchel<sup>28</sup> of which part of the description and one illustration (his Fig. 1) have some resemblance to the tumors under discussion. Another is one of the two parotid adenomas briefly described by Blair and Olch.<sup>3</sup> The possible acceptance of one of these is based on the fact that Gruenfeld and Jorstad,<sup>26</sup> who had an opportunity of examining the cases of Blair and Olch, stated that "one of the two specimens was found to be an exact replica" of the tumor they reported. McFarland<sup>24,25</sup> mentioned another case that probably is a tumor of this type. He included it, together with his case reported in 1927, under the title "Hürthle cell tumor (onchocytoma)." However, he mentioned only that the patient was a female, 74 years old, who had had a tumor in the left parotid gland for many years. The tumor was excised, but he did not describe or illustrate it. Another possible case, because of the description given, is the one

reported by Duplay<sup>20</sup> which was examined by M. Ravier, but was not illustrated. Of the cases included in the group without adequate justification are two adenomas reported by Stöhr and Risak,<sup>30</sup> another reported by Franssen,<sup>31</sup> and one more reported by Steinhardt.<sup>11</sup> The cases of Stöhr and Risak should not be included for the reasons already mentioned by Harris<sup>27</sup>; Franssen's case, about which Lloyd<sup>8</sup> said "there seems no good reason for not including this tumor among the oncocytomas," cannot be accepted because neither the description nor the illustrations is characteristic of the tumors here discussed. Steinhardt's case cannot be included because I believe that this case represents a different type, of which I have had an opportunity to study a few examples that proved to be unlike the ones described in this communication.

Tumors formed by "onkocytes" have been reported in some other organs besides the salivary gland. Hamperl<sup>20</sup> summarized those cases and listed the pancreas, thyroid and parathyroid glands, and hypophysis as sites in which they have been described.

#### OXYPHILIC GRANULAR CELLS IN NORMAL PAROTID GLANDS

From the review of the available literature, it is evident that despite the statement<sup>20</sup> that "onkocytes" have been found in the parotid gland, no definite proof has been given of their demonstration in normal parotid glands. This made apparent the necessity of finding out if they are present in the parotid gland and if they form foci of nodular hyperplasia similar to those described in the other salivary glands. Their demonstration was considered especially important since, with one exception, all of the salivary gland tumors composed of "onkocytes" occurred in the parotid. It is the purpose of this communication to summarize the results of the search for "onkocytes" in 100 parotid glands and to report 5 new cases of parotid adenoma believed to correspond to the group here reviewed.

The 100 parotid glands were obtained from 51 persons on whom autopsy was performed in the University Hospital (Michigan). These were unselected persons who died from various diseases or from accident or violence. Their ages ranged from 7 months to 76 years. Twenty-nine were under 50 years of age, 17 between 50 and 70 years, and 5 were over 70 years.

Most of the material was fixed in 10 per cent formalin, but absolute alcohol also was used in some cases. The sections were stained routinely with hematoxylin and eosin, and Mayer's mucicarmine stain was done in every case in which oxyphilic granular cells were found. Best's carmine stain was used also in the few instances in which the alcohol-fixed material showed the cells under study.

Oxyphilic granular cells were found in 9 of the 51 persons from whom material was studied (Table I). They were present in both parotid glands in 8, and from the ninth only one parotid gland was obtained. The cells were found in ducts, acini, or both, and they were usually seen in several areas of the sections studied from each case. Some of the ducts lined by oxyphilic granular cells were dilated and contained oxyphilic granular material (Figs. 1 to 4); others showed infolding of their walls (Fig. 3). In 4 of the 9 persons the oxyphilic granular cells

TABLE I

*Cases in Which Oxyphilic Granular Cells Were Found in the Parotid Glands after Necropsy*

No.	Sex	Age	Cause of death	State of the parotid glands	Remarks about oxyphilic granular cells
1	M	68	Carcinoma of the stomach	Slight atrophy	Lining ducts
2	F	74	Carcinoma of the breast	Slight atrophy	Transition of duct epithelium into oxyphilic granular cells; lining ducts, and showing adenomatous hyperplasia
3	F	67	Chronic lymphatic leukemia	Moderate atrophy	Lining ducts
4	M	49	Chronic myelogenous leukemia	Marked atrophy	Lining ducts
5	F	64	Third degree burns (carcinoma of the thyroid)	Slight atrophy	In acini
6	M	60	Lobar pneumonia	Marked atrophy	In ducts and acini, with adenomatous hyperplasia
7	F	70	Carcinoma of the thyroid	Marked atrophy	In ducts and acini, forming adenomatous hyperplasia
8	F	75	Carcinoma of the stomach	Moderate atrophy	In acini
9*	M	50	Bullet wounds	Normal	In ducts and acini, with adenomatous hyperplasia

\* Only one parotid gland was obtained.

were forming areas of "hyperplasia" of the adenomatous type (Figs. 1 and 2). Sometimes two or three areas of "hyperplasia" were seen in different fields in one section.

In order to avoid unnecessary repetition, the detailed description of the oxyphilic granular cells will be given in the microscopic description of the adenoma of case 1, but it should be stated that both Mayer's mucicarmine and Best's carmine stains failed to reveal mucin or glycogen in the oxyphilic granular cells. From Table I it can be seen that the youngest person in whom oxyphilic granular cells were found was 49 years old and the oldest 75 years; neither sex was preponderant, and granular atrophy was not particularly associated with the occurrence of such cells except in the youngest person in whom there was a marked atrophy. Areas of "hyperplasia" formed by oxyphilic granular cells were more frequent in this series than in the one of Steinhardt,<sup>11</sup> in which only 2 examples of hyperplasia were found in the salivary glands

of 65 persons. The list of causes of death shows that with two exceptions oxyphilic granular cells were present in persons with a neoplastic process. This relationship may not be significant since the cells occur in a period of life when neoplasms are commonly found. Also, the necropsy service from which this material was obtained is heavily weighted with cases of neoplastic disease. A study of a larger and more representative series is necessary to evaluate this relationship, which was not brought out in Hamperl's study.<sup>15</sup>

## REPORT OF CASES OF ADENOMA

### *Case 1*

The patient (Memorial Hospital accession no. V9578) was a 50-year-old male who gave the following history at the time of admission: Two and one-half years previously he had noted a small area of fullness in the upper part of his neck just below the lobe of the left ear. This area did not appear to grow until 6 to 8 months before admission, after which it slowly increased in size. During the last 3 months the swelling had extended to the pre-auricular and post-auricular regions. There had been no pain or discomfort. One month prior to admission, a small pea-sized nodule appeared in the post-auricular fold. It was tender, red, and spontaneously drained sanguineous fluid about 2 weeks later. After that it decreased markedly in size and tenderness.

On examination there was a soft, somewhat rubbery mass, 4.5 cm. in diameter, in the region of the tail of the parotid gland, just below the lobe of the left ear. The tumor was nontender and nonfluctuant. The posterior margin of the mass was in the post-auricular region. Immediately superior to the mass in the skin of the post-auricular fold there was a 6 mm. reddish nodule which had the gross characteristics of a recently infected "sebaceous cyst." The superior portion of the parotid gland was normal to palpation. Pressure over the mass caused clear saliva to drain from the orifice of Stensen's duct. There was no regional adenopathy or notable intra-oral lesion. The tumor was aspirated and the report upon the material was "salivary gland and lymphocytes." The possibility of Mikulicz' disease was considered and a small amount of radiation was given, which caused no change in the tumor. The material obtained from a second aspiration showed "salivary gland tissue only." Because of the persistence of a well encapsulated, freely movable tumor, the patient was admitted to the hospital for surgical treatment. The surgeon found a lobulated, well encapsulated, pinkish tumor about 3.5 cm. in diameter in the tail of the parotid gland; grossly, the tumor looked like three matted hyperplastic nodes. The patient made an uneventful recovery, with no evidence of recurrence 16 months after operation.

The tissue removed surgically consisted of several nodules resembling lymph nodes in fatty tissue. The largest measured 3.5 by 1 by 1.5 cm.; another was 1.5 by 1 by 1 cm., and four small ones had an average diameter of 6 mm. They were soft, fleshy, and brownish gray on section.

Sections from each of the nodules examined showed a similar appearance. The nodules were surrounded by an incomplete, thin, connective tissue capsule, outside of which there was a small amount of normal parotid gland, which was present also at an area where the capsule was absent. The tumor was composed of lobes separated by connective tissue.

The lobes were divided into irregular lobules by thin fibrous strands. There was some fatty tissue, particularly about the periphery of the tumor and within the normal parotid gland. Blood vessels of medium size were seen throughout. The lobules were formed by masses of epithelial cells arranged in a solid fashion, forming cords composed of single or double rows of cells (Figs. 6 and 11) or adopting a tubular or acinar pattern (Fig. 10). The epithelial cells lay on a thin basement membrane which in some cases was indistinguishable from the walls of capillary blood vessels running between the cords of cells (Fig. 6). The cells were large, spherical, oval, columnar, pyramidal, or polyhedral depending on the pattern which they formed. Their boundaries were clearly seen when they formed cords, acini, or tubules, but were difficult to make out in the solid masses. The cytoplasm was abundant, granular, and stained intensely with eosin (Fig. 6). The granules were small, regular in size, and stained reddish with Masson's trichrome stain and purplish blue with Mallory's aniline blue. A fine reticulated structure could be made out in some of the cells in which the granules were in the spaces of the network. The nuclei were small, round or oval, with fine granular chromatin evenly distributed. They had a prominent nucleolus, and sometimes two nucleoli were seen. Some of the cells had two nuclei or an elongated nucleus with a constricted area in its middle portion. Throughout the sections there were some cells which stained more intensely. They were elongated, pyramidal, or irregular, with dark, irregularly indented nuclei in which no nucleoli could be seen. Their more eosinophilic cytoplasm contained slightly larger granules. These cells often were in pairs or in small groups.

The ducts were markedly reduced in number and only a few could be considered normal. Some of the excretory ducts were distended with coarsely granular substance in which a few leukocytes and desquamated cells were seen (Fig. 7). A few ducts showed flattened epithelium which progressively changed into a cuboidal type with the characteristic oxyphilic cells. When studied in serial sections, one branched duct with the features previously described showed the cells of its ramifications to be continuous with cords of tumor cells (Fig. 7). In some instances the striated ducts, lined with eosinophilic cells, were seen also to form a unit with cords of tumor cells, all of them resting on a continuous basal membrane.

Some of the intercalated ducts were lined by stratified squamous epithelium and ended in glandular structures. These glandular formations had a basal membrane on which rested a row of low-cuboidal or flattened epithelial cells; toward the center the cells increased in size and became vacuolated. These cells had central nuclei; the cells closer to

the lumina of the ducts had irregular pyknotic nuclei. They were seen also in the lumina of some of the ducts as if forming a holocrine secretion. The structures were similar in all respects to sebaceous glands. These glands were observed also to arise from striated ducts in a few instances.

There was an area of parotid tissue in one section, between two lobules of tumor, in which, in addition to the normal acini with cells containing bluish or purplish blue granular cytoplasm, there were other acini, the cells of which stained from light pink to a marked eosin red. Such acini seemed to represent different stages of transformation from normal acini into those of the fully developed adenomatous type. The acini which showed the earliest change were of normal size but the granules of their cells were eosinophilic. A narrow, clear halo could be seen around each nucleus (Fig. 8). In a more advanced stage, cells and acini were larger than is normal. The cells had a more eosinophilic granular cytoplasm and the clear halos around the nuclei were still present in some of the cells (Fig. 9). When the changes were more advanced, the acini were formed by cells with all of the characteristics of those in the tumor, except that they were slightly smaller (Fig. 10). In this stage, a few of the cells showed a very fine, faint, perinuclear halo. In all of these stages the nuclei showed no noticeable change. The different features described in the acini were seen to occur also in the intercalated ducts, which in some cases contained a granular eosinophilic substance in their lumina.

Throughout the sections there were lymphocytes forming irregular groups but without follicle formation. They were frequently seen around ducts and they were more numerous in these areas (Fig. 7). There was no evidence of the presence of any of the components of the "mixed tumors." Careful search of the sections failed to reveal cells undergoing mitosis.

### *Case 2*

The patient was a female, 72 years old, who was admitted to the University Hospital with the chief complaint of a swelling in the right side of the neck, present for 7 to 10 years. When discovered it was the size of a small walnut, but it had increased during the last 4 to 5 years. There were no other symptoms.

On examination there was an elongated tumor, soft and uniform in consistency. It extended from the right mastoid and parotid regions downward for a distance of about 6 cm. The tumor was movable, nontender, and measured about 4 cm. in width. It was removed surgically and the surgeon stated that it was well encapsulated, lobulated, and soft. It extended from the level of the hyoid bone to a point 2 cm. above and behind the angle of the jaw. The tumor was easily stripped from its capsule after several large vessels passing through it had been clamped and divided. There was mild facial palsy after the operation. The patient recovered uneventfully and her palsy completely disappeared. She was without evidence of recurrence 12 years and 8 months after operation.

The tissue removed surgically (University of Michigan, no. 1406-AN) was a flat, lobulated tumor measuring 8 by 5 by 2.5 cm. with a uniformly firm consistency. On section it showed a dark brown, lobulated surface.

Microscopic sections revealed a lobulated tumor surrounded by a thin fibrous capsule. The lobes were separated by septa which in some areas resembled those of a normal parotid gland. A small amount of non-neoplastic parotid gland was seen outside the capsule and also inside of it about the periphery of the tumor (Fig. 14). The parotid tissue just inside the capsule was greatly compressed. There were many thin-walled blood vessels in all sections. This tumor was similar in appearance to that of case 1, with areas of solid and loose structure. There was fatty tissue throughout the section, but it was more abundant in the less solid areas. Normal ducts were absent from all of the sections. The few ducts present were seen in the interlobar septa. They were lined by stratified squamous epithelium and one of them contained desquamated epithelium in its lumen. There was lymphocytic infiltration around some of the ducts; there were groups of lymphocytes in other areas of the tumor also, but they never formed germinal centers. The tumor cells, like those in case 1, were arranged in cords, acini, tubules, and gland formation (Figs. 12 and 13). The cellular details were similar to those of the previous case except for areas in which the granular appearance of the cytoplasm was not so apparent. The granularity of the cytoplasm was best observed in sections stained with Mallory's aniline blue. The nuclei had the same characteristics as in case 1, although pyknotic nuclei were not present in cells with the more eosinophilic cytoplasm, a type of cell rarely seen in this case. Some cells were binucleate. At the periphery of the tumor there was an area of dilated ducts lined by cells of the same type as those seen in the tumor. Some of the ducts contained an eosinophilic granular material (Fig. 14). The same substance occasionally was seen in the lumina of duct-like structures throughout the tumor. The normal parotid tissue showed areas in which the acinar cells were slightly eosinophilic, suggesting the earliest state of transformation into tumor, as described in case 1. Study of numerous sections failed to show any myxomatous, cartilaginous, or other tissues usually seen in the so-called "mixed tumors" of the parotid gland. Mitotic figures were not seen in any section.

### *Case 3*

There was no clinical history available for this case. Two slides (no. 7507-LAV) were sent to the Laboratory of Pathology of the University of Michigan with the statement that they were "from an encapsulated, largely necrotic tumor of the parotid region."



The tumor might have been about 2 cm. in diameter, as judged by the sections submitted. The sections were stained with hematoxylin and eosin. One of them was restained with Weigert's iron hematoxylin.

One of the sections showed a lobulated tumor rimmed by a fibrous capsule. Outside the capsule there were some salivary gland acini and ducts surrounded by proliferating fibroblastic tissue and inflammatory cells. The remainder of the section showed almost complete necrosis except in the interlobar septa which were infiltrated by leukocytes. In the other section the tumor was encapsulated. It showed a compact structure with areas of necrosis. There were some thin-walled blood vessels, more numerous in the center of the section. The cells of the tumor were spherical, oval, or polygonal; they formed a solid mass with some suggestion of arrangement in cords and acini (Fig. 15). The cells rested on a basement membrane and stained intensely with eosin. In general, cellular detail was not very clear, but there were some areas in which the cells showed the characteristic appearance described for case 1. Their nuclei were dark, small, round, or oval, with fine granular chromatin and a single nucleolus. As in the 2 previous cases, there were none of the components usually found in "mixed tumors," and no evidence of mitotic division.

#### *Case 4*

The patient was a female, 60 years old, who had an abscess in the left parotid region in 1927. She was told that it was due to infected tonsils, and tonsillectomy and drainage of the abscess were done. In 1943 she noted a small tumor at the angle of the left jaw. This tumor grew slowly and on one occasion it was excised and drained, but it never disappeared.

On physical examination there was a firm, irregular, oval-shaped mass, measuring about 4.5 by 2.5 cm., just behind and slightly below the angle of the left jaw. The tumor was excised. At operation it appeared to be fairly well encapsulated and was the size of a large walnut. The tumor was found in the inner prolongation of the parotid gland, and there was no evidence of infiltration.

The only material available from this case was a prepared slide stained with hematoxylin and eosin (University of Michigan, no. 8325-LAX). The two sections on the slide showed an encapsulated tumor with interlobular septa. Outside of the capsule there was a small amount of normal parotid gland in which two of the intercalated ducts showed glandular structures indistinguishable from sebaceous glands and similar to those observed in case 1. In both sections there was an area of dense fibrous connective tissue in which some salivary ducts, distended blood vessels, collections of lymphocytes, and a few macrophages containing blood pigment were present. This area probably represented scar tissue resulting from the previous excision and drainage. The tumor was formed by

solid masses of cells bounded by a basement membrane and arranged in cords, lobules, and alveoli. The cells were round, oval, or polygonal and had a finely granular eosinophilic cytoplasm. The nuclei were like those in the cases previously described (Fig. 16). No mitotic figures or indications of a mixed tumor were seen.

### Case 5

This tumor (University of Michigan, no. 4856-LAO) was submitted on March 6, 1937, without clinical history except that it was from the parotid gland of a male. On January 20, 1948, it was learned that the patient was without evidence of recurrence and that he had been 32 years old at the time of operation in 1937.

The size of the specimen was not recorded but it probably was about 1.5 cm. in diameter, judging by the dimension of the sections. Microscopic examination showed that the tumor was lobulated and encapsulated, with a small amount of normal parotid gland attached to the capsule. Septa of fibrous tissue separated the lobules. The tumor showed areas of compact structure, into some of which hemorrhage had occurred. In the solid areas the tumor mostly formed cords of cells arranged in single or double rows. There also were areas of acinar or glandular formation (Figs. 18 and 19). In the areas of hemorrhage the cords had been broken up, giving the tumor a pseudo-papillary appearance (Fig. 19). The tumor cells varied in shape from spherical to columnar. Other cellular details were the same as described for the other cases, except that cells with two nuclei were seen more frequently (Fig. 17). Study of many sections failed to reveal a myxomatous or chondromatous component. A few mitotic figures were present.

### DISCUSSION

A definite answer to the question in respect to the significance of oxyphilic granular cells cannot be given at the present time. Each investigator has advanced his opinion. Schaffer<sup>12</sup> considered them as a form of degeneration of the salivary gland epithelium. Pischinger<sup>13</sup> interpreted them as undifferentiated cells which, by amitotic division and further development, substituted for the destroyed glandular elements. Zimmermann<sup>14</sup> considered them as anomalous cells because they were not present in all persons. He also expressed the opinion that they probably were an abnormal form of cellular differentiation. Hamperl<sup>15,16,20</sup> interpreted them as a peculiar irreversible form of atrophy of the epithelium which also, in a broad sense, could be considered as degeneration. He emphasized the fact that the cells under discussion were associated with old age and glandular atrophy. Škorpil,<sup>10</sup> on Hamperl's findings

and his own, considered them to be normal in old persons and to represent an irreversible transformation of the epithelial cells into a new type. However, it is still unknown why that change takes place, and what the activities of this new type of cell may be. The presence of eosinophilic granular material in the lumina of some of the ducts, resembling the granules in the cytoplasm, suggests that they probably are able to secrete, or at least to extrude, part of their cytoplasmic content. This seems to be true both in the cases in which the cells show "adenomatous" hyperplasia and in neoplasms in which they assume a glandular structure.

Another problem with which one is confronted is to decide whether the tumors formed by these cells should be considered as true neoplasms or as a form of hyperplasia. The problem is made still more difficult by the fact that the cells involved in the process are not the normal cells of the gland, but result from the transformation of normal cells into cells of an entirely new type. The best way to answer the question is to try to reconstruct the process that may take place. Normally, for unknown reasons, but probably in relation to advanced age and glandular atrophy, the cells of some ducts, acini, or both, undergo transformation into the new type, forming isolated groups, or, in other cases, giving origin to areas of "nodular hyperplasia" which may be of solid, adenomatous, or adenomatous and papillary character.<sup>10,11,15,16</sup> On the other hand, usually in old persons but not always so and again for unknown reasons, the transformation of the glandular epithelium does not remain localized but extends progressively to most of the elements around the original area of epithelial transformation, or it may spread from newly formed areas. At the same time, proliferation of the cells results in enlargement of the gland. This enlargement can be explained, first, by increase in size of all of the cells involved in the process, since they are larger than the ones from which they arise, and secondly, by cellular proliferation, which in many cases does not seem to be marked, since the evidence of cellular division is limited. The result is the formation of a new structure sometimes presenting a glandular appearance, but which no longer is parotid gland or has any resemblance to it. In summary, the oxyphilic granular cells form a new and abnormal structure which arises from the gland by transformation of its cells into a new type and involves an increase in size of the area affected. Thus it seems reasonable to conclude that the process represents neoplasia rather than glandular hyperplasia.

Sometimes this tumor formation may originate in various areas of "adenomatous hyperplasia" in a single parotid gland. This occurrence may take place simultaneously or at different times, which explains the multicentric origin of some of the tumors reported, such as those of

Gruenfeld and Jorstad,<sup>26</sup> Harris,<sup>27</sup> and case 1 of this communication, all of which were formed in several nodules. It also explains the "recurrence" of the tumor reported by McFarland,<sup>9,22,23,25</sup> as has already been pointed out by Ackerman.<sup>6</sup> In trying to explain the origin of the tumor, Gruenfeld and Jorstad, Harris, and Škorpil<sup>10</sup> have already demonstrated that the ducts take part. In their opinions, the tumor originates from them. This is true in some cases. However, it is also true that the transformation of the cells can occur first in the acini, and in such circumstances, if a tumor develops, its origin should be considered as being from the acini. There seems to be enough evidence to substantiate the belief that all epithelial elements of the gland, with the possible exception of those of some of the excretory ducts, are liable to undergo transformation into the new cells and therefore to form the tumor. Evidence that acinar cells take part in the formation of the tumor is found in the changes observed in such cells in case 1 (Figs. 8 to 10), and to a lesser degree in case 2 of this report. The participation of the acinar component in the formation of tumor seems not to have been observed before, although it could have been expected on the basis of the findings in normal glands.

The presence of sebaceous glands, observed in ducts of cases 1 and 4, will be discussed in another communication,<sup>32</sup> but it should be stated now that such sebaceous glands form no part of the tumor. Their occurrence was entirely incidental.

This tumor, although rare, has characteristic features by which it can be easily recognized: It is composed of peculiar oxyphilic granular cells, which some authors have compared to the cells of the liver or adrenal<sup>9,20,26,27</sup>; normal ducts are absent or diminished; and there are groups of lymphocytes which do not form follicles. This tumor may present an adenomatous form with acinar and tubular formations as in cases 1 and 2, or it may adopt a more solid appearance with few areas of acinar or tubular structure, as in cases 3, 4, and 5 of this report. In the more solid form, the groups of lymphocytes may be few and small.

This tumor has been described or listed under different names, such as "onkocyte tumor," "oncocyoma," "onkocitäres adenoma," and "Hürthle cell tumor (onchocyoma)." In addition, the name "pyknocyoma" has been proposed. The term most commonly used—"oncocyoma" or "onkocyoma"—was first proposed by Jaffé<sup>33</sup> to designate a tumor of the parotid region more widely known by the names "papillary cystadenoma lymphomatosum" or "adenolymphoma." The term "oncocyoma" obviously is based on the name "onkocyte," given by Hamperl<sup>15</sup> to the cells herein described. This term "onkocyte," although probably appropriate for the reasons advanced by Hamperl,<sup>15,20</sup> has proved to be

confusing since the Greek root *ὄγκος* has been used extensively to form other medical terms with the connotation "pertaining to tumor." Interpretation of "onkocyte" as "tumor cell" is entirely different from the idea of large size which Hamperl tried to convey by this word.

It is apparent that the term "oncocytoma" should not be used to name the tumor under discussion since it is not only an unfortunate term, but it was originally devised to designate a different tumor. The term "pyknocytoma" suggested by Harris<sup>27</sup> does not seem appropriate because it uses the word "pyknocyte" already discarded by Hamperl<sup>15</sup> for reasons which he gave, and because it implies that the tumor is composed of pyknotic cells, which is not correct. Therefore, it is necessary to devise new terms which will designate more accurately these cells and the tumors formed by them, and which at the same time will not create further confusion. With this idea in mind I believe that a descriptive term such as "oxyphilic granular cells" should be used to designate the cells under discussion until future studies determine their exact nature and significance. For the same reasons a similar descriptive term should be used to name the tumor formed by these cells. "Oxyphilic granular cell adenoma of the parotid gland" or "oxyphilic adenoma of the parotid gland" can be used to designate the neoplasms composed of these cells and of the type described in this communication.

#### SUMMARY

One hundred parotid glands from 51 persons were investigated for oxyphilic granular cells (oncocytes). These cells were found in 9 persons who ranged from 49 to 75 years of age. In 4 individuals the cells produced nodular hyperplasia, and in the remaining 5 they were present in the ducts or acini.

In connection with the description of 5 new cases of oxyphilic granular cell adenoma of the parotid gland (oncocytoma), their origin has been traced to cells of both ducts and acini which undergo transformation into the neoplastic type. The descriptive terms "oxyphilic granular cell" and "oxyphilic granular cell adenoma" are proposed to replace the confusing designations of "oncocytes" and "oncocytoma."

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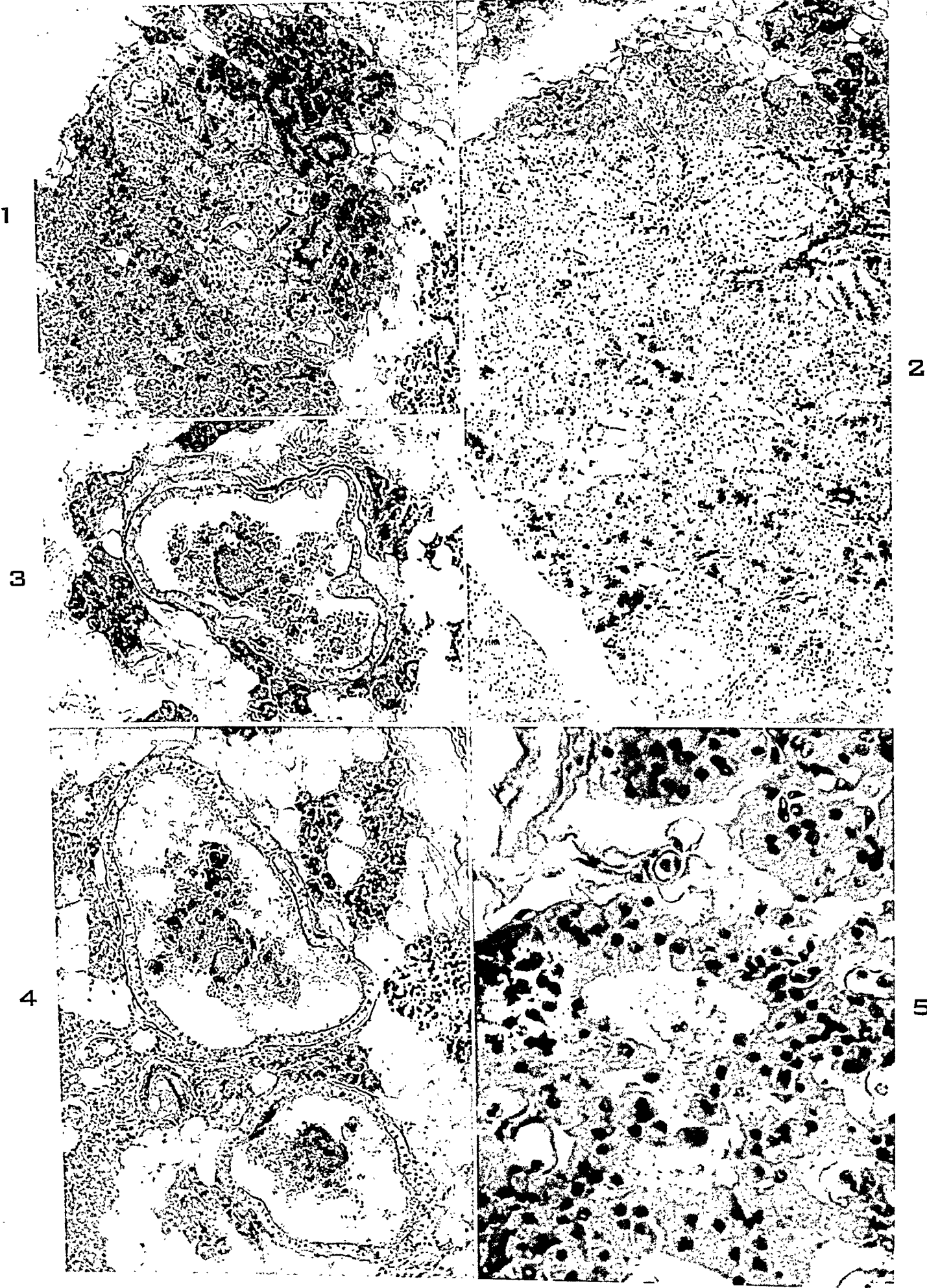
### DESCRIPTION OF PLATES

All photomicrographs were obtained from slides stained with hematoxylin and eosin, unless otherwise stated.

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#### PLATE 76

- FIGS. 1 and 2. Case 2, Table I. Oxyphilic granular cells forming "adenomatous hyperplasia" in a normal parotid gland. There is a dilated duct lined by oxyphilic granular cells in the lower portion of Figure 2.  $\times 115$ .
- FIGS. 3 and 4. Case 4, Table I. Normal parotid gland, showing dilated ducts lined by oxyphilic granular cells. Of note are the granular material in the lumina of the ducts and the infolding of the wall of the duct in Figure 3.  $\times 115$ .
- FIG. 5. Case 7, Table I. Oxyphilic granular cells from another area of hyperplasia in an otherwise normal parotid gland.  $\times 375$ .



Meza-Chávez

Oxyphilic Adenoma of Parotid Gland

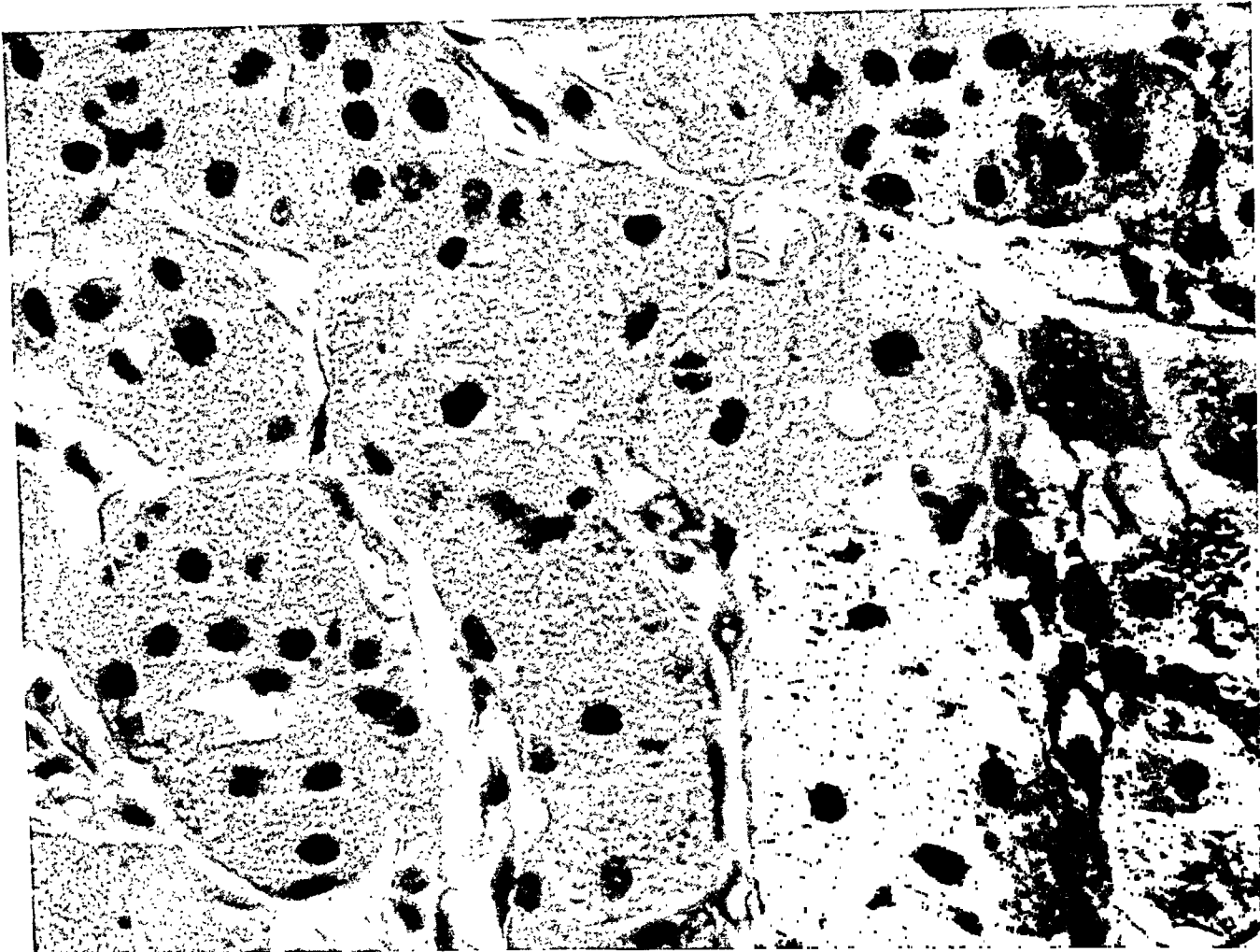


PLATE 77

FIG. 6. Case 1. Area from the adenoma of the parotid gland in which cords of cells are seen as well as the characteristic cellular structure of the oxyphilic granular cells. Trichrome stain.  $\times 650$ .

FIG. 7. Case 1. Distended duct containing oxyphilic granular material in its lumen and lined by oxyphilic granular cells in all its branches. Trichrome stain.  $\times 100$ .

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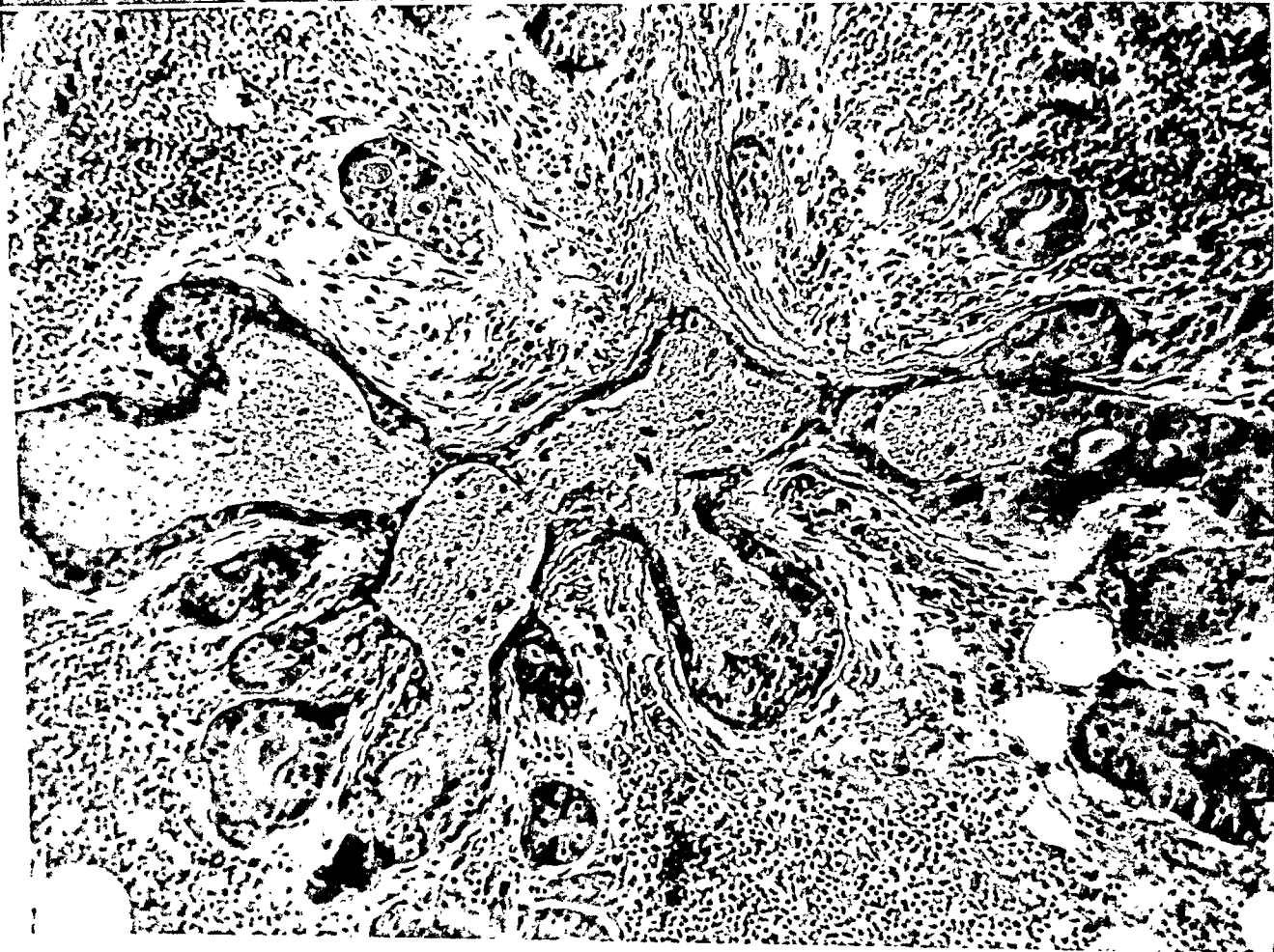


PLATE 78

FIGS. 8 to 10. Case 1. Three stages of transformation of parotid epithelium into oxyphilic granular cells as described on page 530.  $\times 375$ .

FIG. 11. Case 1. Area of the neoplasm in which the cells are forming cords composed of single and double rows of cells.  $\times 125$ .

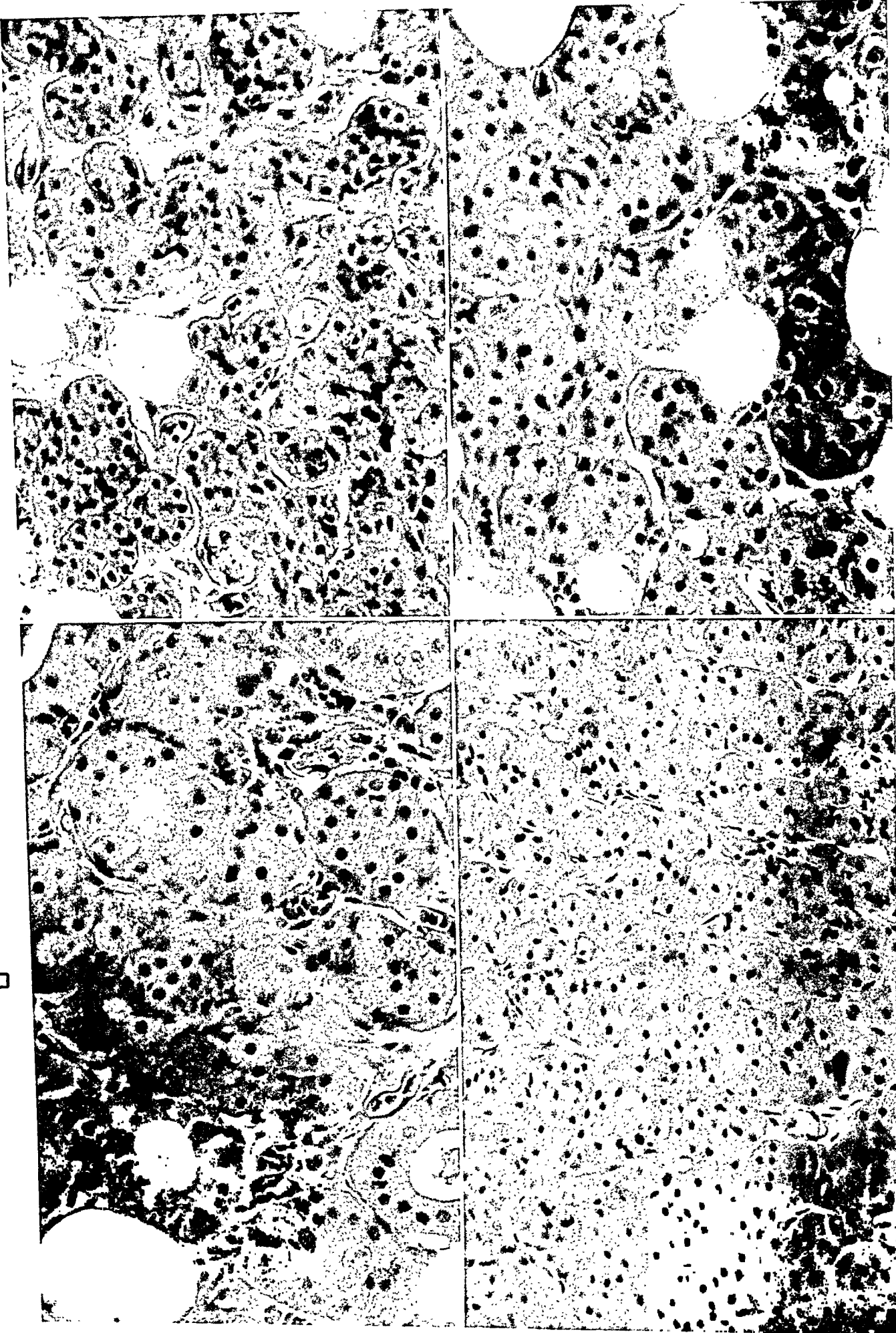
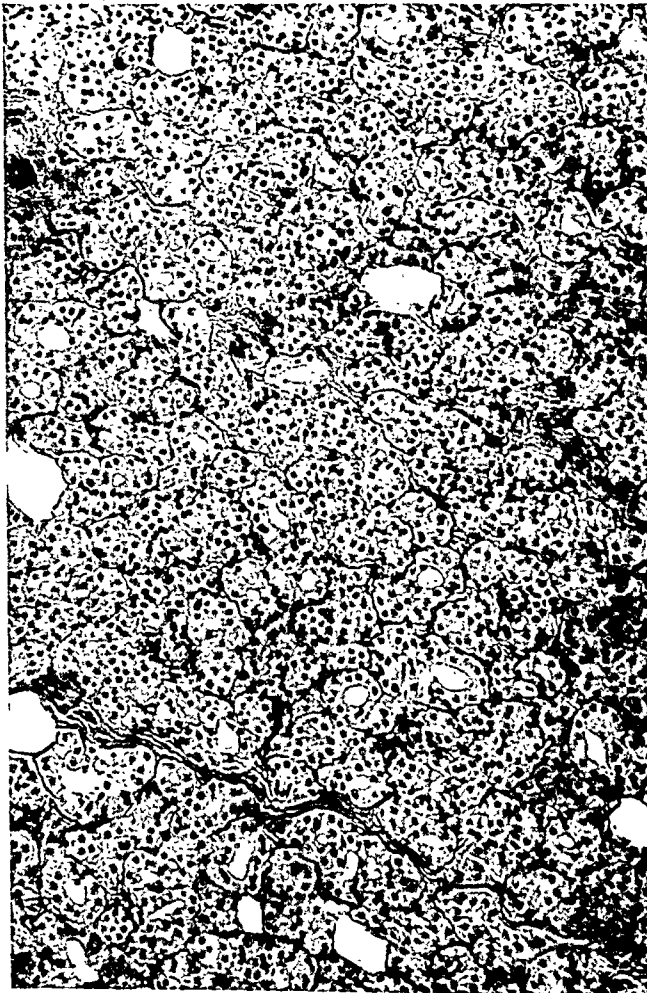


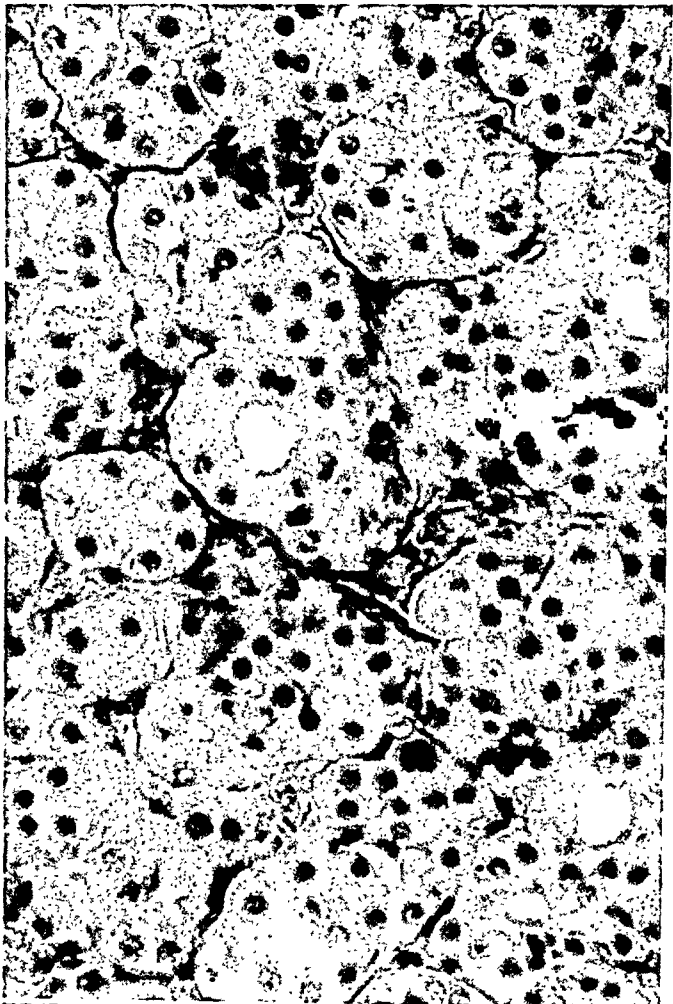
PLATE 79

- FIG. 12. Case 2. Parotid adenoma. Area of the tumor showing a tubular and acinar structure.  $\times 115$ .
- FIG. 13. Case 2. Parotid adenoma. Tubular and acinar structure seen at higher magnification.  $\times 375$ .
- FIG. 14. Case 2. Parotid adenoma. Of note are the distended ducts lined by oxyphilic granular cells, the infolding of the wall of one of the ducts, and the non-neoplastic parotid gland about the periphery of the tumor.  $\times 115$ .
- FIG. 15. Case 3. Area from parotid adenoma. Weigert's iron hematoxylin and eosin stain.  $\times 115$ .

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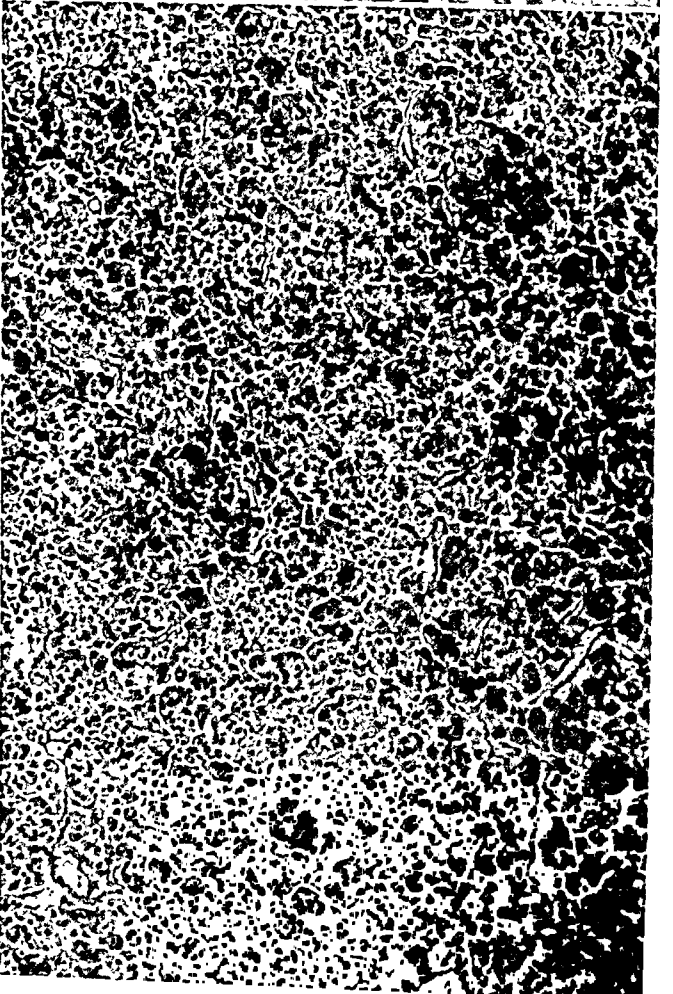
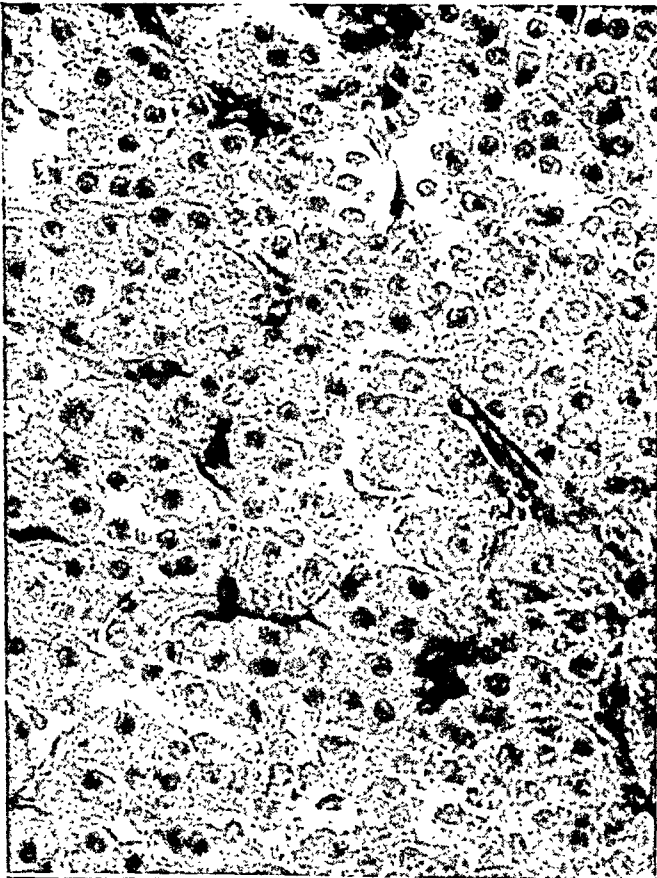


PLATE 80

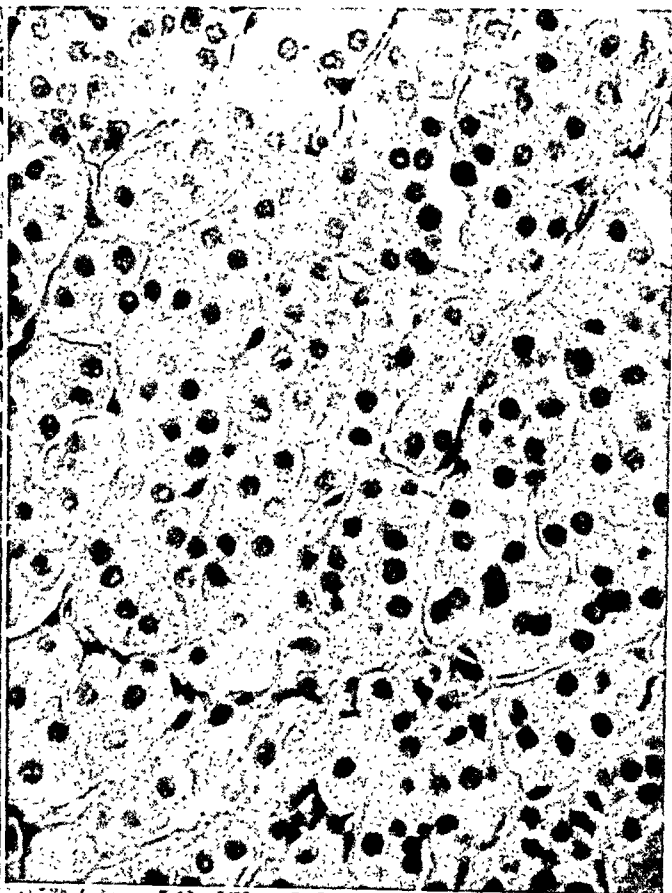
- FIG. 16. Case 4. Area from adenoma of the parotid gland, showing the characteristic appearance of the oxyphilic granular cells.  $\times 375$ .
- FIG. 17. Case 5. Parotid adenoma. In this area the tumor has a solid appearance, and there are several binucleated cells.  $\times 375$ .
- FIG. 18. Case 5. Normal parotid gland outside the tumor capsule. The neoplasm shows a mixed solid and acinar structure.  $\times 115$ .
- FIG. 19. Case 5. Portion of parotid adenoma showing a pseudo-papillary appearance.  $\times 115$ .



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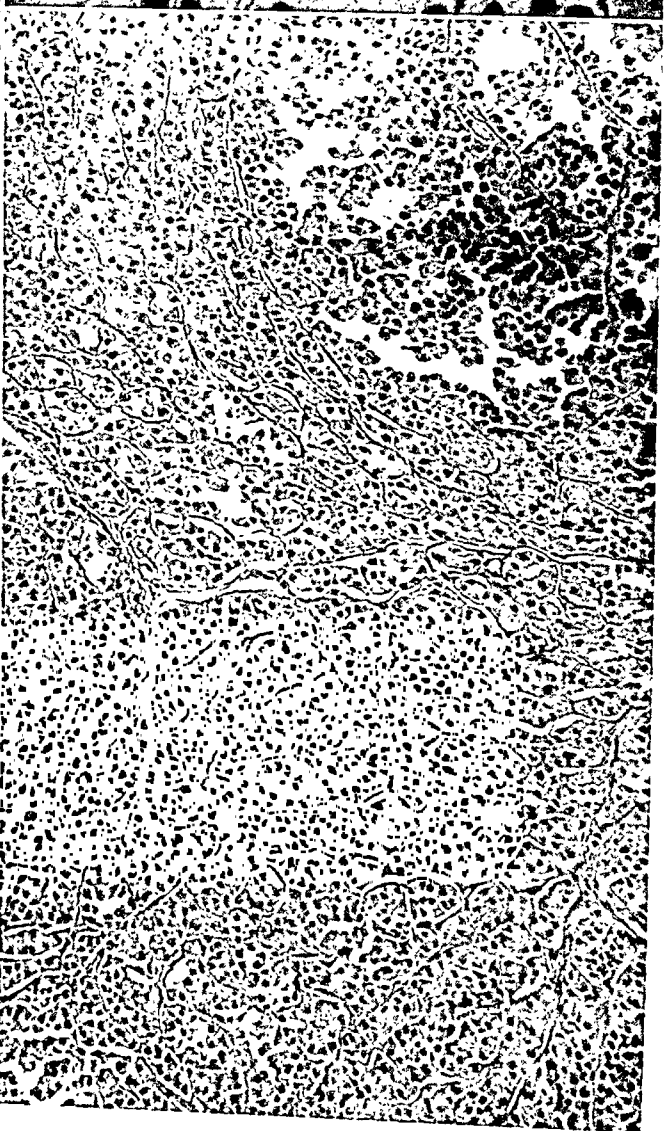
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## INTRAMURAL FIBROMA OF THE HEART \*

WILLIAM KULKA, M.D.

*(From the Cuyahoga County Coroner's Office, Cleveland, Ohio)*

Tumors of the heart of all types are rare.<sup>1</sup> As the majority of such tumors are asymptomatic, or at least without specific signs during life, they interest only the pathologist. However, some can be the cause of cardiac dysfunction or even cardiac failure and death, and thus can become of some concern to the clinician. It is assumed that the reason for the frequent lack of serious cardiac dysfunction even in the presence of extensive neoplastic involvement of the heart is due chiefly to the lack of invasion of the valves or to the slow development of the growth, giving time for compensatory changes.<sup>2</sup>

In the exhaustive studies and reports by Mönckeberg,<sup>3</sup> Mandelstamm,<sup>4</sup> and Yater<sup>2</sup> of all neoplasms of the heart which they could collect from their own cases and in a search of the literature, they mentioned only 2 which could be considered as fibroids<sup>3</sup> or intramural fibromas.<sup>2</sup> One had been described by Luschka<sup>5</sup> in 1855, the other by Albers<sup>6</sup> in 1856. Yater, however, questioned that even these 2 cases should be classified as true fibromas.

Luschka<sup>5</sup> found the tumor which he reported in the wall of the left cardiac ventricle of a 6-year-old boy who had died with diphtheria. He described the tumor as similar in aspect and consistency to a fibromyoma of the uterus. A definite capsule could not be discovered. The tumor measured 4.3 by 3.5 cm. Histologically, it consisted of a meshwork of interwoven columns and whorls of connective fibers and collagenous substance surrounded by, and embedded in, the cardiac musculature.

Albers<sup>6</sup> described tumors which he called chondroid. One of them he presented as early as 1833.<sup>7</sup> The second one<sup>6</sup> was discovered incidentally during an autopsy on a 77-year-old man whose previous history remained unknown. In this later case there was a carious process and formation of a cold abscess at the site of the 3rd to 5th thoracic vertebrae which communicated through a fistula-like channel along the ribs, with a carious process in the manubrium sterni. The left ventricle was densely adherent to the pericardium over an area of 5 by 5 cm., wherein was enclosed a hard, grayish white mass of the same size. This growth bulged 4 to 5 mm. above the surface of the heart and was separated from the endocardium by a layer of cardiac musculature 3 to 4 mm. in thickness. In the center there were only a few capillaries, but at the periphery

\* Received for publication, May 20, 1948.

there was a network of vessels. In addition, Albers found in this same body a second tumor, the size of a hen's egg, which was located outside the pericardial sac adherent to the ascending aorta. On cross section, this tumor was grayish white; it was fibrotic at the periphery and showed a cheese-like necrosis in the center. From this description, it may be strongly questioned whether this last case of Albers could be classified as a fibroma of the heart.

The extreme rarity of confirmed fibromas of the heart without involvement of the valves and auricles, as evidenced by the very meager literature, prompted me to present the following case.

### REPORT OF CASE

A white female infant, 8 months old and apparently in good health, died suddenly under the following circumstances: The father had played with the child and left her alone for a few minutes, returning hurriedly when he heard her cough and found her dyspneic. The baby, who had been fed about 15 minutes prior to the attack, developed hiccups and severe cyanosis. Immediate artificial respiration and administration of oxygen were unsuccessful and she died before arrival at the hospital. This sudden and unexplained death automatically became a problem for the coroner's office.

*Previous History.* The baby had been delivered at term by cesarean section because of cephalic-pelvic disproportion. The mother had been under progesterone treatment during the second and third months of pregnancy because of threatened abortion. The baby weighed 7 lbs. at birth and seemed well developed. She was the second child in this family; her 11-year-old brother, also delivered by cesarean section, is well developed and in good health. Other living relatives in good health include both parents, three grandparents, and one great grandfather who is 90 years of age. The death of the maternal grandfather at the age of 72 was the result of an accident.

The baby ate well and gained weight under artificial feeding, sat up freely, and had begun to stand up in the crib. She slept well, cried little, and was of a cheerful disposition. Some choking or vomiting following drinking of water or milk was considered within normal limits. Hiccups were habitual after feeding. There were occasional attacks of what the parents described as "quivering sensations" (*i.e.*, slight tremorous movements) during which both hands were stretched out and the eyes rolled in all directions. These attacks lasted only a short time and the child would be normal afterwards. The physician who attended the baby at birth and saw her four times afterwards for physical checkup and inoculation found no signs of abnormality.

### AUTOPSY FINDINGS

At autopsy (no. M 2737), the body was that of a well developed white female baby, 28 inches in length and weighing 22 lbs. Ecchymoses were seen in the reddened conjunctivae. The lips were cyanotic and there was some vomited material in the oral cavity and in the larynx. Further examination revealed that the lower medial incisors had erupted recently and that the upper medial incisors were in process of erupting. The anterior fontanelle measured 1 cm. in diameter. The external aspects of the head, neck, chest, abdomen, and extremities were of normal appear-

ance. There were ecchymoses, subpleural in the thymus, and in both lungs. The lungs also showed acute congestion. The thymus measured 11 by 6 by 1 cm.

*Heart.* The heart (Fig. 1) weighed 60 gm. The right ventricle measured 5.5 cm. in length, 5.5 cm. wide at the base, and the thickness of the wall on cross section was 4 to 5 mm. The left ventricle measured 6 cm. in length and was 5 cm. wide at the base. Embedded in the anterior wall of the left ventricle was a hard, tumor-like mass, 5 cm. in vertical diameter and 3.5 cm. in its horizontal diameter. It extended from the anterior part of the septum through the anterior wall to the lateral margin of the left ventricle, and upward close to the annulus fibrosus of the left ventricle. The aortic and mitral valves and papillary muscles were not involved in the tumor and were of average size.

The right ventricle and right auricle were dilated. The foramen ovale and ductus arteriosus were closed. The aorta and pulmonary artery were of average size.

On cross section the hard mass was grayish white, glistening, slightly bulging, and nodular, resembling a myofibroma of the uterus. A thin layer of cardiac musculature covered its periphery. On the inside it adjoined the endocardium. The apex of the heart was not involved. No definite capsule separated the mass from the surrounding muscle tissue.

*Lungs.* Both lungs were acutely congested. There were a few ecchymoses in the pleura. The larynx and trachea contained some aspirated gastric material.

*Spleen.* The spleen was enlarged and congested.

*Stomach.* The stomach was filled with semi-digested material, cheesy particles, and yellowish cloudy fluid.

*Liver.* The liver weighed 400 gm. and was engorged with blood.

All other organs appeared somewhat congested but otherwise were of a size and appearance consistent with the age.

### *Microscopic Findings*

Upon microscopic examination the tumor was seen to consist of interwoven columns of fibroblasts with well stained spindle-shaped nuclei and acidophilic cytoplasm. There was some collagenous stroma and, rarely, a thin-walled capillary (Figs. 2 and 3). Mitotic figures were inconspicuous. In the periphery of the mass, where the number of cells was markedly decreased, a stroma of collagenous material and connective fibers prevailed. This material extended between the overlying muscle fibers. No separating capsule-like layer could be seen. In sections stained by van Gieson's picric acid and acid fuchsin technic for demon-

stration of collagen and reticulum, a dense network of delicate fibrils was seen in the central areas; in the periphery of the tumor there were fibers of varied size and thickness with an amorphous collagenous substance interlaced with the cardiac muscle fibers. To obtain optimal photographic results, sections were stained also according to Mallory's phosphotungstic technic (Fig. 4).

#### COMMENT

These macroscopic and microscopic findings identify this tumor as a fibroma of a peculiar type. It differs decidedly from the so-called polypoid tumors of the heart and even more so from the characteristic features of myxoma.<sup>1,2,8</sup>

Taking into consideration the location of the tumor and its histologic characteristics, there seems to be basis for the assumption that it was derived from elements of the embryonic reticular jelly that lies between the primitive endocardium and epimyocardium.<sup>9</sup>

Although there were no significant attacks noted during life, it may be concluded, from the tremendous dilatation of the heart and thinning of the musculature of the left ventricle, that the tumor played a decisive rôle in the death since the reserve power of the heart must have been greatly reduced. The sudden paroxysm of coughing, vomiting, and aspiration of gastric material proved to be a greater load than this heart could sustain.

Whether the treatment of the mother with progesterone during pregnancy or the necessary roentgenologic examinations because of the cephalic-pelvic disproportion have a bearing on the formation of this congenital tumor is problematic.

#### SUMMARY

Upon autopsy of an 8-months-old child, who had died suddenly, a tumor not involving the valves was found in the wall of the left cardiac ventricle, causing marked dilatation of the heart. The tumor, measuring 5.5 by 3.5 cm. in diameter, had the macroscopic and microscopic characteristics of an intramural fibroma.

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[ *Illustrations follow* ]

## DESCRIPTION OF PLATES

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### PLATE 81

FIG. 1. The left cardiac ventricle showing the tumor mass, the thinned layer of cardiac musculature, and the intact papillary muscles and mitral valve. The intact aortic valve is behind the tumor.  $\times 1$ .

FIG. 2. Central area of the cardiac fibroma. Hematoxylin and eosin stain.  $\times 70$ .

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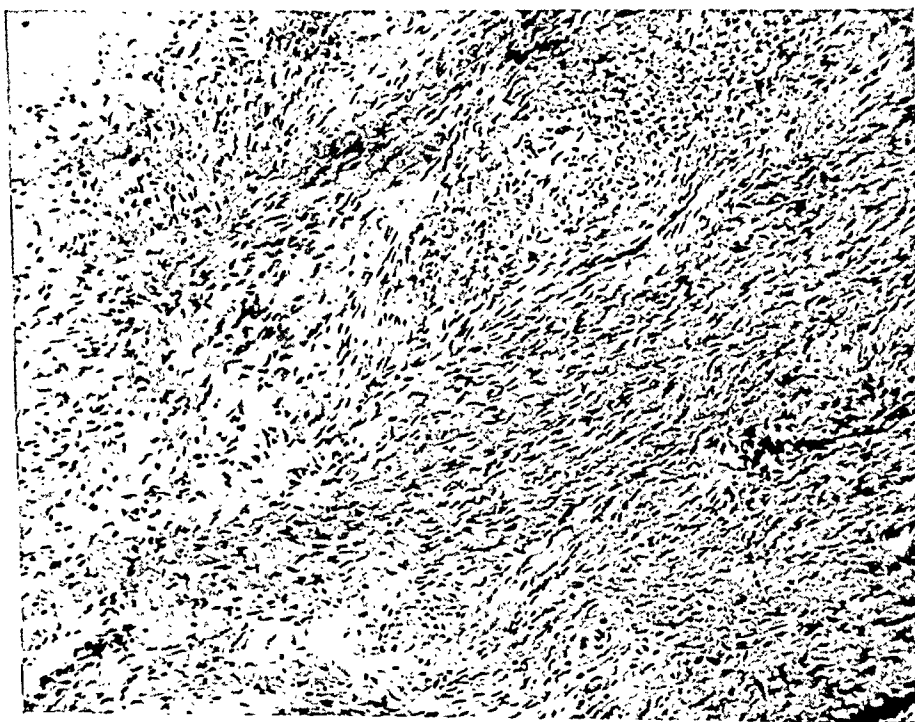


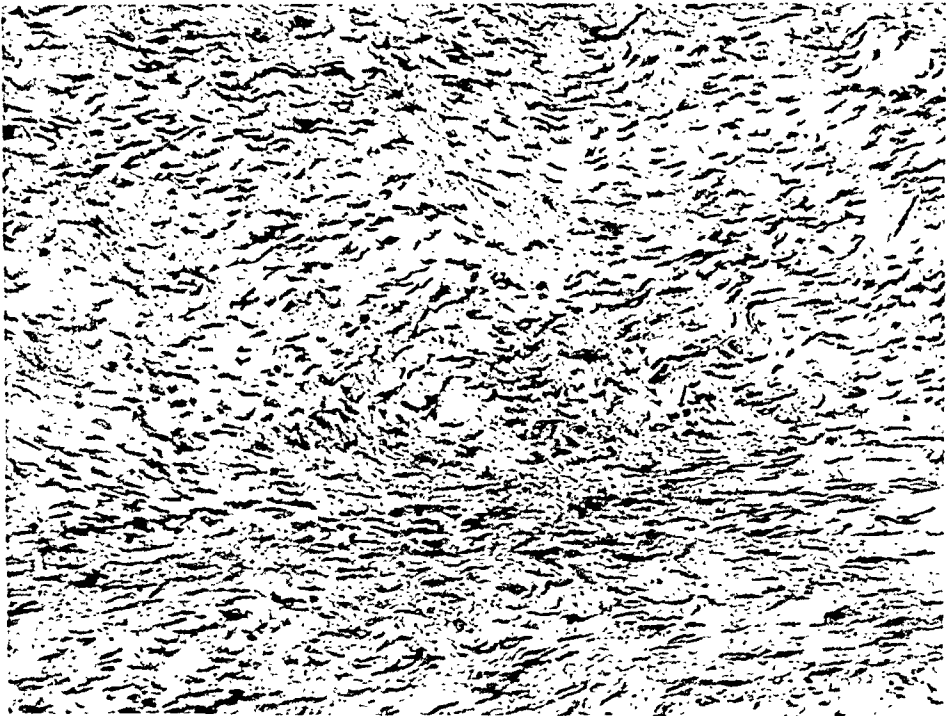


PLATE 82

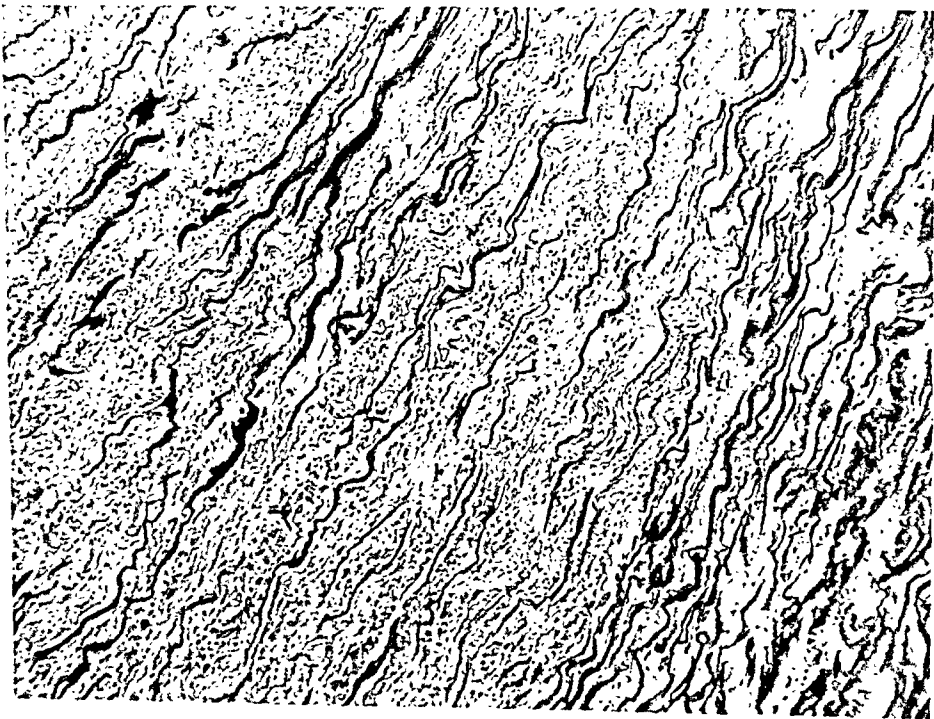
FIG. 3. Arrangement of fibroblasts in the center of the tumor. Hematoxylin and eosin stain.  $\times 150$ .

FIG. 4. Periphery of the tumor, showing collagenous fibrils intermingled with the dark-stained cardiac muscle fibers. Phosphotungstic acid-hematoxylin stain.  $\times 70$ .

3



4



Kulka

Intramural Fibroma of the Heart



## MUCORMYCOSIS OF THE LARGE BOWEL \*

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Fungi of the family Mucoraceae, usually referred to as molds and commonly encountered as contaminants in routine culture and as saprophytes on dead or decaying vegetation, may occasionally produce infection in man. The genus *Mucor* has been responsible for a large number of lesions in man with perhaps *M. corymbifer* as the best known species. Gregory, Golden, and Haymaker<sup>1</sup> reviewed the literature and noted that in mucormycosis usually only a single organ or system is involved, most commonly the lungs or the ears. Superficial mucormycosis is not common, with only a few reported instances. Serious infection with *Mucor* likewise is rare although within recent years several instances have been revealed, including 4 in the central nervous system.<sup>1,2</sup>

The case herein reported appears to be unique in that it was characterized by mucosal inflammation and multiple ulcerative lesions of the large bowel. The lesions were infected uniformly by a fungus which appeared morphologically to be a *Mucor*. The involvement was extensive, actively inflammatory, and led to perforation of the bowel with death from generalized peritonitis. Although gastric ulcers infected with these fungi have been reported,<sup>1</sup> no previous record has been found of a widespread ulcerative colitis due to *Mucor*.

### REPORT OF CASE

*Clinical History.*† The patient was a white woman, 37 years old, who was admitted to St. Joseph's Hospital, Milwaukee, complaining of generalized abdominal pain, nausea, and vomiting. Her symptoms began a week prior to admission with severe cramp-like pain in the left flank, followed by the passage of a hard mucus-covered stool. During the following days, the stools became looser, were tan, and contained large quantities of mucus. No gross blood was reported. The family physician made a diagnosis of "intestinal flu" and placed her on sulfonamide medication. The abdominal pain became generalized at the end of a week and was accompanied by nausea and vomiting.

The patient gave a history of having suffered from recurring attacks of transient gastro-intestinal disturbances during the previous 5 years. They occurred several times each year and were accompanied by the passage of mucus-covered stools. A physician had not been consulted. From her own experience she avoided greasy and fatty foods because of the distress that they caused. There was no clinical or laboratory evidence of diabetes mellitus.

On admission the patient was acutely ill. Her abdomen was noticeably distended

\* Received for publication, June 4, 1948.

† We are indebted to Drs. E. G. Collins and W. Casper for the clinical information on this case.

and tender. Bowel sounds were absent. The white blood cell count was 13,300, with 21 per cent band cells, 56 per cent segmented neutrophils, 14 per cent lymphocytes, and 9 per cent monocytes. The urine contained occasional leukocytes, 1 or 2 red blood cells per high-power field, and a few crystals. A roentgenogram of the abdomen revealed a ladder-like arrangement of the loops of the small bowel and suggested intestinal obstruction or ileus. The impression after physical examination was that an acute, spreading peritonitis was present, due to a ruptured appendix or diverticulum. Surgical procedures were not attempted and a conservative course of treatment was instituted, consisting of chemotherapy, intravenous fluids, blood transfusions, and Wangensteen suction. The patient's condition rapidly became worse and she expired 8 days after admission to the hospital.

#### NECROPSY FINDINGS

At necropsy, 3 hours after death, the abdomen was still greatly distended but contained no palpable masses. The omentum covered the abdominal viscera and was bound firmly within the pelvis. When the omentum was released, thick, gray fluid, amounting to approximately 500 cc., welled upward into the abdominal opening. Other localized areas containing lesser amounts of purulent exudate were found throughout the abdominal cavity. A large pocket of purulent material, lying between the spleen and diaphragm, extended upward behind the pleura into the chest wall. The small bowel was dilated and its loops were bound together by recent fibrinous adhesions. The serosal surfaces of all abdominal organs were dull and appeared thickened. The appendix was not ruptured. A small rounded perforation, measuring 0.4 cm. in diameter, was discovered in the inferior surface of the cecum. The large bowel displayed numerous small, shallow, irregular, mucosal ulcers. The ulcers were present throughout the entire length of the colon and varied from approximately 0.5 to 2.0 cm. in diameter. The bases of the ulcers were pinkish white and were comparatively free from exudate. One of the ulcers had extended completely through the wall of the cecum, forming the small perforation noted above.

The spleen was soft and its capsule was thickened, especially where it formed the floor of an abscess. The pulp was soft and easily scraped from the cut surface. The remaining abdominal organs and tissues showed no significant abnormalities.

The left lung was partially collapsed and the pleural cavity contained approximately 500 cc. of clear yellow fluid. A retropleural abscess was found in the left posterior thoracic wall. It communicated with the abdomen by passing beneath the diaphragm.

Permission was not granted to examine the central nervous system.

#### *Microscopic Examination*

On microscopic examination, the epicardium was thickened and infiltrated with lymphocytes and plasma cells. A similar change was noted

in the visceral pleura, which was covered also with a thin coat of fibrin. The pulmonary alveolar spaces and the small bronchioles contained pink-staining fluid and occasional polymorphonuclear leukocytes. The splenic capsule was greatly thickened and infiltrated with plasma cells and lymphocytes. The splenic pulp was congested and contained numerous neutrophilic leukocytes. No pertinent changes were found in the liver, adrenals, kidneys, pancreas and internal genitalia.

*Gastro-intestinal Tract.* Multiple sections taken through the shallow ulcers of the colon presented identical microscopic appearances. The serosa was edematous, infiltrated with leukocytes, and its surface was covered with a thick, inflammatory exudate consisting of interlacing strands of fibrin and entrapped degenerating leukocytes. Lymphocytes, plasma cells, and occasional neutrophils were found scattered through the muscularis. The submucosa was three times its usual thickness, due to intense edema and an accumulation of mononuclear cells. The mucosa was edematous and in many areas necrotic. The glands and stroma in many areas were broken up into a granular mass of débris. Scattered among the necrotic tissues and in the bases of the ulcerative area were large accumulations of polymorphonuclear leukocytes and filaments of a fungus. Disruption of the muscularis mucosae and extension into the submucosa frequently were found. The same inflammatory changes encircled the fungus in the submucosa. At several points the fungus had invaded the small vessels of the submucosa and could be seen within their lumina. The vessel walls showed early necrotic changes and were infiltrated with polymorphonuclear leukocytes. In most areas extension did not occur more deeply than into the submucosa. Careful microscopic examination failed to reveal the fungus in the peritoneal exudate, in the lungs, or in other organs.

### *Mycologic Findings*

The Mucors in general, like the Aspergilli and the Penicillia, are considered to be the weeds of mycology. They are found as saprophytes growing on dead or decaying plants or animals. Since these organisms produce numerous spores which are easily disseminated by the wind—air-borne—it is not uncommon to find them on food that we ingest or in the air that we breathe. Occasionally, these fungi may become parasitic or pathogenic as primary agents of disease in man. Usually, however, they attack man as secondary invaders, being dependent upon a preceding alteration of the tissues by infection by other organisms, chiefly bacteria, as in the case of tuberculosis; by general or constitutional disorders; by various tumefactions, notably neoplasms; or by direct trauma.

In the routine cultivation of skin lesions of various types and of sputa, it is not unusual to find these organisms as contaminants. On occasion one may feel reasonably sure that these fungi play a rôle, either major or minor, in pathogenesis. *Aspergillus* is the most frequent culprit, followed closely by *Penicillium*. *Mucor* is not a frequent offender but appears in a sufficient number of sputum cultures to harass the mycologist. When found producing single isolated colonies, they are usually passed over lightly. When found in abundance, however, they should be considered seriously.

It is unfortunate that cultures of the fungus responsible for the lesion described in this case were not made before the material had been placed in a fixative. The diagnosis of mucormycosis may be established with a degree of certainty on the basis of characteristics both of the tissue reaction and of the various morphologic variations of the fungus in its parasitic phase in tissue. Under these circumstances, in the absence of actively growing cultures, the organism can be identified only as to genus and not as to species. Several cases of mucormycosis, with and without the cultivation of the fungus on artificial media, have appeared in the literature. The authors agree on the general morphologic properties of the fungus in tissues, which are those of a species of *Mucor*; namely, that the organism has a comparatively large diameter, varying in different tissues; branching of the filaments, either abundant or not; lack of septa—coenocytic hyphae; and large accumulations of filaments in the affected tissue.

The appearance and location of the fungus in the tissue are closely linked to the state and degree of tissue reaction. The general picture of mucormycosis as observed in the sections of this case was that of an intense inflammatory process with massive areas of edema and necrosis (Fig. 1). At the periphery of the inflammatory zone was a sharp demarcation between normal and affected mucosa. Here the fungi were not seen until the area was reached where the mucosa began to show a reaction. The fungous filaments were few, appeared isolated, and were enlarged, irregular, or sclerotic. In the distinctly inflamed and hyperplastic zone the fungi were of a smaller diameter and seemed to penetrate the tissue toward the unaffected region. The filaments appeared to be very active, showing numerous branching forms which had little regard for barriers since they had invaded the lumina of the glands and had branched freely (Fig. 2). The same held true for the blood vessels, with the filaments having branched within the lumina (Fig. 4).

In the necrotic areas the filaments were abundant either as isolated large forms or as freely branching growths (Fig. 5). The hyphae ap-

peared uniform in size with actively growing, lightly staining tips indicative of young protoplasm. Cross-wall formation or septa were not seen. The presence of lightly stained areas in the filaments—vacuoles—adjacent to intensely stained segments gave at times the appearance of septum formation. In the sections, groups of filaments could be seen which on cross section gave the appearance of spores (Fig. 7). This peculiar picture was due in many cases to the irregular branching and kinking of the filaments. This characteristic was particularly noted in nodular growths in which the fungous filaments were intertwined and matted together with various inflammatory tissue cells (Figs. 3 and 6).

In summary, it may be said that although cultures of the fungus were not obtained, thus making absolute identification impossible, a sufficient number of characteristics were readily visible to suggest a close relationship to the Mucoraceae. The large size of the filaments, both in diameter and length, the method of free and irregular branching, and the absence of cross-wall formation all point to Mucor. To strengthen this view, one may refer to the publications of others in which it was stated that cultures were obtained, and that the organism, in addition to possessing the above properties, produced similar tissue changes with invasion of the lumina of the intestinal glands and of the blood vessels.

#### SUMMARY

In a patient with acute colitis due to a fungus, there were ulcerative colonic lesions, perforation of the bowel, and death from generalized peritonitis. The morphologic features of the fungus, as found in the lesions produced by it, indicated that it was in all probability a species of Mucor.

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[ *Illustrations follow* ]



### DESCRIPTION OF PLATES

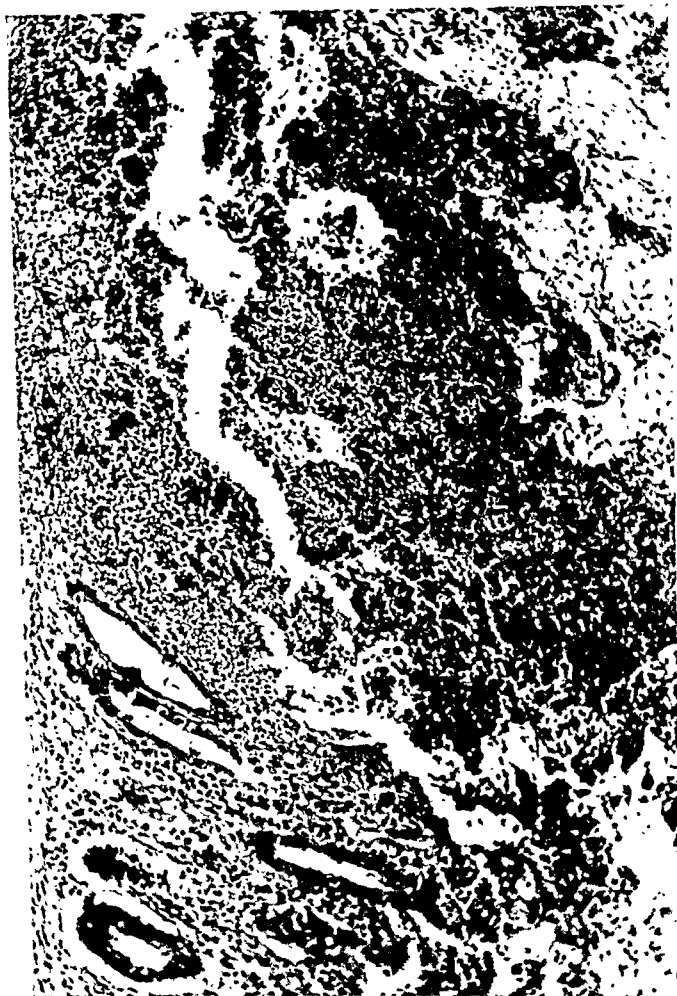
All photomicrographs were made from sections stained with hematoxylin and eosin.

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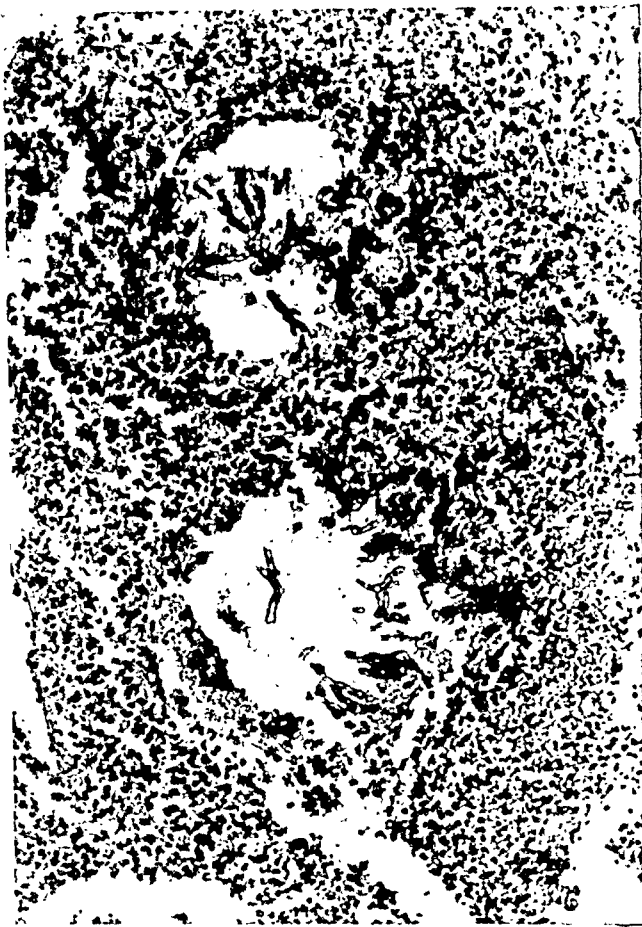
#### PLATE 83

- FIG. 1. Section through an inflammatory and necrotic zone of the mucosa.  $\times 90$ .  
FIG. 2. An inflammatory and necrotic area, showing branching fungous filaments in the lumina of the destroyed glands.  $\times 165$ .  
FIG. 3. Nodule of intertwined filaments and inflammatory cells in the submucosa.  $\times 50$ .  
FIG. 4. Blood vessel invaded by the fungus, which has branched.  $\times 715$ .

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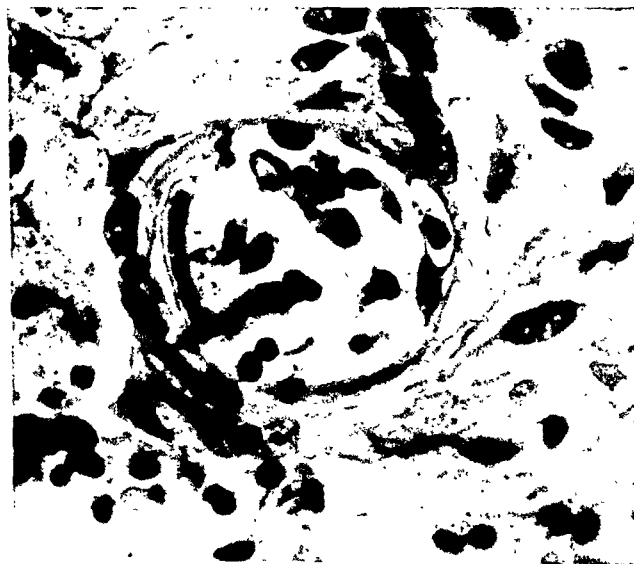
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Moore, Anderson, and Everett

Mucormycosis of the Bowel

PLATE 84

FIG. 5. Freely branching forms of the fungus in a necrotic area, showing vacuolar structure and young, growing tips of filaments.  $\times 515$ .

FIG. 6. Nodule of an intertwined mass of fungous filaments and inflammatory cells. Many of the hyphae appear in cross-sectional form.  $\times 120$ .

FIG. 7. Fungous filaments seen in cross section. Their resemblance to spores may be noted.  $\times 545$ .

FIG. 8. Young filament invading the tissue. Intense tissue response is absent.  $\times 685$ .

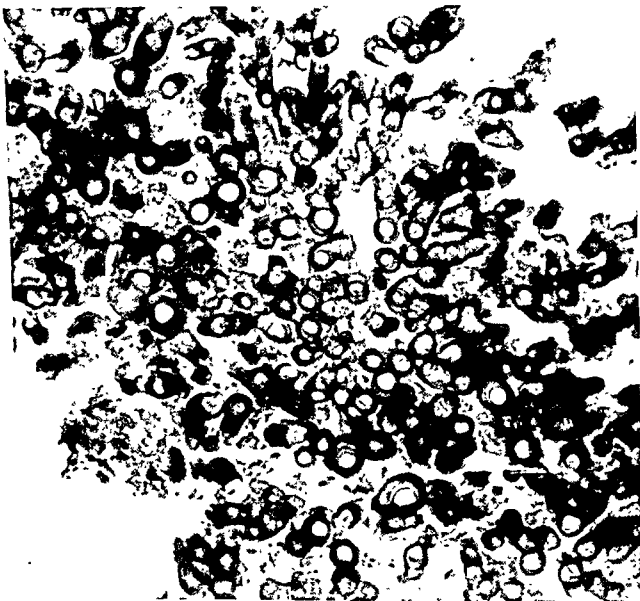
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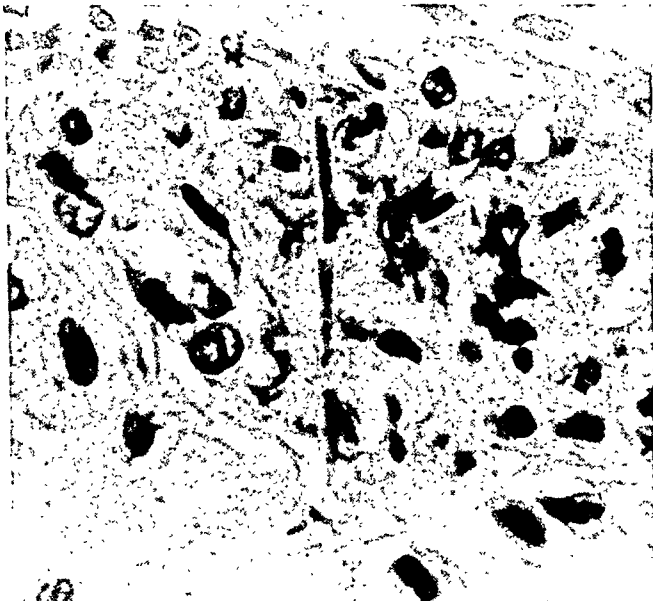
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Mucormycosis of the Bowel



## CANINE TOXOPLASMOSIS \*

R. F. LANGHAM, D.V.M., and L. B. SHOLL, D.V.M.

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Toxoplasmosis in the dog appears to be extremely rare. Mello<sup>1</sup> in Italy, in 1910, described the first case. The symptoms observed in this animal were anorexia, weakness, anemia, emaciation, dry and firm skin, poor development of the skeleton, atrophy of the muscles, respiratory disturbances, diarrhea, vomiting, and a weak pulse.

Carini,<sup>2</sup> in 1911, upon microscopic examination of the lungs, spleen, liver, kidneys, and bone marrow of a dog, was able to demonstrate organisms having the characteristics of *Toxoplasma cuniculi*. He was able to reproduce the condition in pigeons.

Yakimoff and Kohl-Yakimoff,<sup>3</sup> in 1911, reported a case in a dog. The injection of an emulsion of bone marrow into 8 white mice, 4 rats, 4 rabbits, 3 dogs, and 2 pigeons produced death in the mice, pigeons, and 2 dogs, and organisms were isolated from the organs.

Nicolau and Kopciowska,<sup>4</sup> in 1935, found the organisms of toxoplasmosis in the brain, bone marrow, spleen, lymph nodes, lungs, small intestine, colon, and cardiac muscle of a dog dead from this disease.

Machattie,<sup>5</sup> in 1939, observed 2 cases of toxoplasmosis in dogs. At autopsy one animal showed marked congestion of the lungs and enlargement of the spleen, while the other exhibited necrosis of the lungs and liver.

Olafson and Monlux,<sup>6</sup> in 1942, reported the first cases in the dog in the United States. In addition to describing the disease in 4 dogs, they also described it in a cat and a sheep. The infection was characterized by a sudden onset, high temperature, extreme depression, and a short course. The tissues most commonly attacked were lymph nodes, liver, lungs, and intestines. The organisms occurred in a variety of cells: Monocytes, hepatic cells, vascular endothelium, smooth muscle cells, and pancreatic epithelium.

Perrin,<sup>7</sup> in 1943, made a study of the protozoan organisms toxoplasma and encephalitozoon, which are easily confused with each other. He injected these organisms into mice, guinea-pigs, hamsters, and rats. He used special stains for bringing out differential characteristics of the two in sections of tissue and smears.

\* Published with the permission of the Director of the Michigan Agricultural Experiment Station as Journal Article No. 971, new series.

Received for publication, June 18, 1948.

Hagan,<sup>8</sup> in 1943, gave a good summary of the disease as it occurs in animals.

The illustrative case which we wish to report is not recent, going back to 1924.

### REPORT OF CASE

A young fox terrier, weighing 10 lbs., was brought to the clinic for examination. Pneumonia was diagnosed and the animal died despite treatment.

### AUTOPSY FINDINGS

At autopsy the lungs were dark red and showed numerous light gray foci. These pneumonic nodules suggested tuberculosis, but no tubercle bacilli were demonstrated. The liver displayed numerous dull-gray, pin-point lesions which, with the congestion, gave it a nutmeg appearance. The mucosa of the stomach was very congested and revealed numerous ulcers, measuring from 1 to 10 mm. in diameter. Several small ulcers were present in the intestinal mucosa. There were several hemorrhages, the largest measuring 12 mm. in diameter. Other organs and tissues were somewhat congested.

### *Histopathologic Findings*

Microscopically, the lungs showed a very acute and severe pneumonia. There was extensive congestion, and a large number of alveolar lumina contained erythrocytes. In other areas there was serofibrinous exudate. The accumulation of fibrin in the alveolar walls and lumina was somewhat patchy. Some polymorphonuclear leukocytes and macrophages were present, with an occasional macrophage revealing cytoplasm filled with toxoplasma. Several giant cells were present. The bronchi and bronchioles showed some desquamation of the epithelium and hemorrhagic exudate in the lumina.

In the liver there were very extensive areas of patchy necrosis (Fig. 1). There were numerous hepatic cells at the edges of the necrotic areas that appeared to be invaded by individual toxoplasma. A few Kupffer cells had large numbers of parasites in the cytoplasm (Fig. 2).

The intestines showed an ulcer extending into the submucosa and surrounded by an inflammatory process. With the necrotic debris were large numbers of polymorphonuclear cells, some lymphocytes, fibrin, and some hemorrhage. Outside of this zone was another characterized by large macrophages, lymphocytes, and some congestion. This inflammatory zone extended through the submucosa, muscle layers, and serosa. The smooth muscle layers showed marked atrophy and necrosis. There were numerous toxoplasma present in smooth muscle cells, and in the

cytoplasm of many macrophages and occasional giant cells (Fig. 3). The walls of some arteries, especially the media, contained them.

The organisms appeared round or oval, and were about 2 to 4  $\mu$  in length and 1.5 to 2.5  $\mu$  in width. Each had an eccentrically placed nucleus, staining deep blue. The cytoplasm was stained uniformly pink or pale blue (hematoxylin and eosin). The toxoplasma were found free, loosely grouped within the cell cytoplasm, or in compact, cyst-like accumulations. It appeared that these cyst-like accumulations were contained usually in the cytoplasm of macrophages or giant cells (Fig. 4). The isolated organisms showed their outlines a little more clearly than did those in the cysts.

After comparing the pathologic findings and the staining characteristics of the parasites of this case with those described by Perrin,<sup>7</sup> we consider the organism to be toxoplasma.

#### SUMMARY

An acutely fatal disease in a dog was characterized by pneumonitis, gastric and intestinal ulcers, and foci of hepatic necrosis. The parasites found in the microscopic sections had the structure and distribution described for *Toxoplasma cunicula*.

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[ Illustrations follow ]



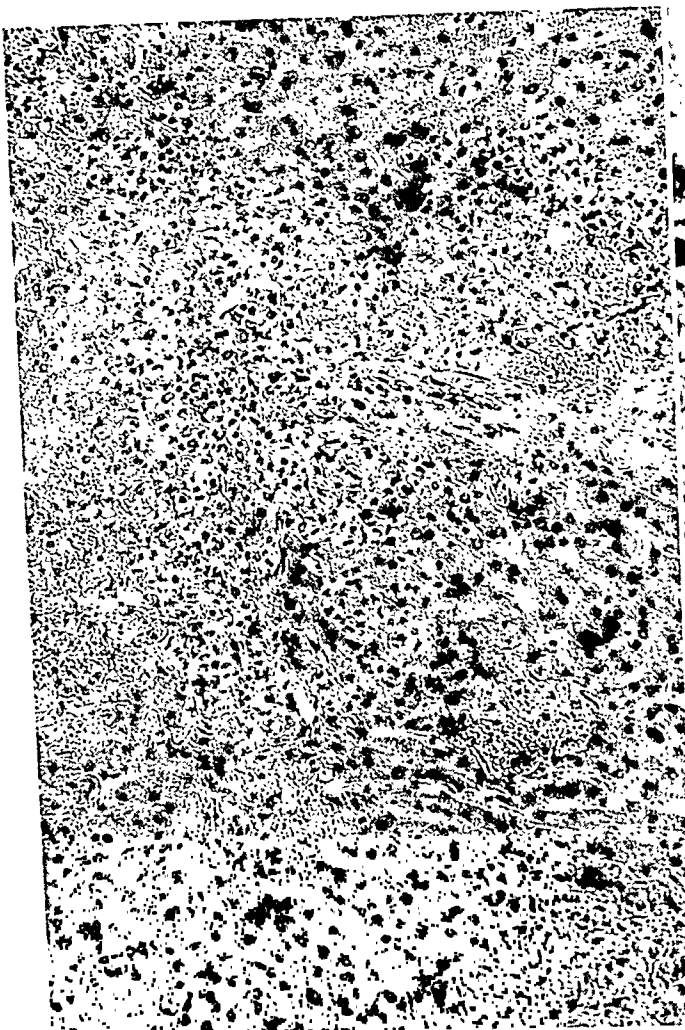
## DESCRIPTION OF PLATE

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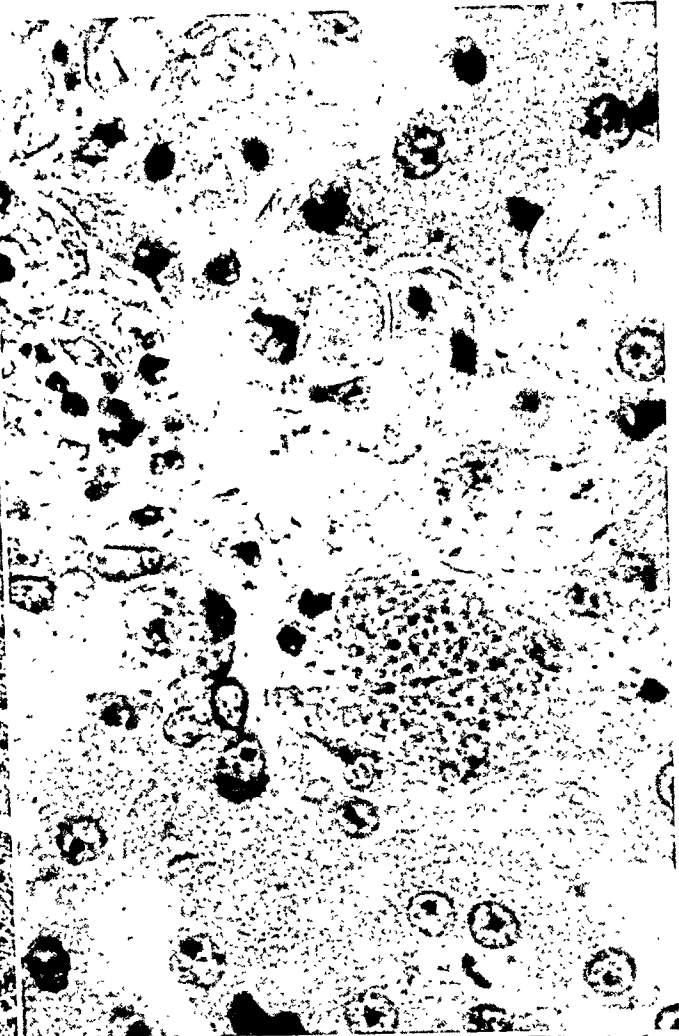
### PLATE 85

- FIG. 1. Liver, showing patchy areas of necrosis. Hematoxylin and eosin stain.  $\times 160$ .
- FIG. 2. In the liver, a macrophage at the margin of a necrotic area is filled with toxoplasma. Hematoxylin and eosin stain.  $\times 780$ .
- FIG. 3. The intestinal wall shows necrosis of the muscle and macrophages containing the organisms. Hematoxylin and eosin stain.  $\times 780$ .
- FIG. 4. A giant cell filled with toxoplasma, as seen in the intestinal muscle. Hematoxylin and eosin stain.  $\times 1240$ .

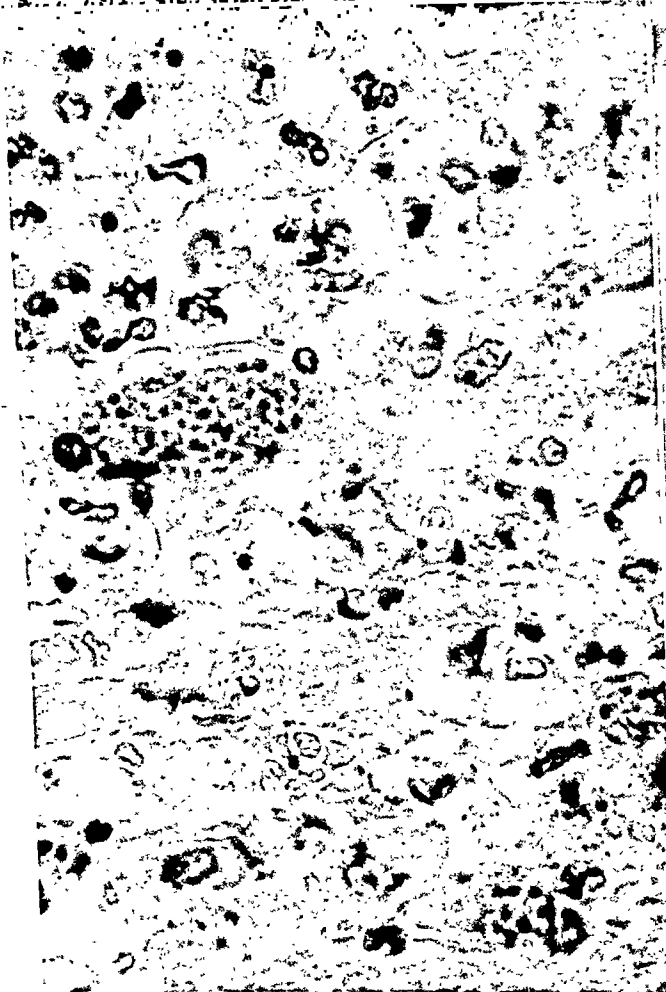
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# THE AMERICAN JOURNAL OF PATHOLOGY

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NUMBER 4

## THE REACTION PRODUCED IN THE PULMONARY ARTERIES BY EMBOLI OF COTTON FIBERS\*

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In the routine examination of sections of lungs from necropsies over a period of 20 years, we have observed, as an incidental finding in 6 cases, foreign bodies in the smaller branches of the pulmonary arteries. These foreign particles were slender and had a faintly greenish tinge; they did not stain with the dyes used in routine histologic preparations and were doubly refractive. They did not cause thrombus formation. The foreign bodies usually lay against the intimal surface of the artery and in some instances a few large mononuclear cells and several multinucleated giant cells of the foreign body type had collected about them (Fig. 1).

When the histories of these patients were studied, there was one feature common to all: Each had received either one or more intravenous injections of physiologic saline or glucose solution or blood transfusions, within a period of not more than 10 days prior to death. The foreign particles were considered to be fragments of cotton that were contained in the solution injected and that had come either from the gauze through which the solution had been filtered, or from particles of a cotton stopper that adhered to the mouth of the flask.

To test the correctness of the impression that these foreign particles were cotton fibers and to study in detail the lesions that might be produced by them in the pulmonary arteries, the following experiments were performed.

### PROCEDURE AND METHODS

A small mass of cotton, either absorbent or nonabsorbent, was immersed in physiologic saline solution and cut into very minute fragments. These were of such a size as to be readily drawn into a syringe through a no. 19 gauge hypodermic needle. Each cubic centimeter of saline solution contained approximately 0.02 cc. of cotton fibers. The physio-

\* Received for publication, July 6, 1948.

logic saline solution containing the cotton fibers was then sterilized in the autoclave at a pressure of 15 lbs. for 30 minutes.

Sixty-two adult albino rats were used in the experiment. Under ether anesthesia, an iliac vein was exposed, and approximately 1 cc. of the saline solution with the suspended cotton fibers was injected. When the injection did not proceed rapidly, it was found that the fibers had settled and jammed the plunger of the syringe. When this occurred another injection was made into the other iliac vein. There were no untoward effects from the injections and each animal appeared normal as soon as it recovered from the anesthesia. Rats in groups of 3 to 5 were sacrificed at varying intervals, some at the end of 24 and 48 hours and others at approximately weekly intervals thereafter for the first 2 months and at longer intervals between the third and sixth months.

At necropsy, 3 or 4 specimens of tissue were obtained from the lungs of each animal; these were fixed in Zenker's solution without the addition of acetic acid or formalin, and in 10 per cent formalin solution. Three to 5 sections were cut from each paraffin-impregnated block of tissue. In some instances, the block was sectioned serially. The routine stains were hematoxylin and eosin, and Weigert's stain for elastic tissue counterstained with hematoxylin and eosin. Occasionally, Wilder's silver stain counterstained with azocarmine-aniline blue was used.

## RESULTS

Some of the animals were injected with absorbent cotton, others with nonabsorbent cotton. The changes observed were the same, and a single description of the results will suffice for both groups. Before discussing the results it is necessary to review briefly the structure of the pulmonary artery of the rat.

The first branches of the pulmonary artery to enter the various lobes of the lung have walls of uniform thickness, the media being well developed. In the branches of medium size, the wall of the artery often undergoes an abrupt change in thickness, owing to a sudden thinning of the media. This change may involve both sides of the vessel as seen in longitudinal or tangential section, although in many the thinning of the media is restricted to a segment of the wall. From these vessels of medium size, branches are at times given off abruptly at right angles. The arterioles have walls of uniform width. The precapillary branches consist of endothelium, a single layer of elastic tissue, and adventitia.

The injected cotton fibers were lodged chiefly in the arterioles and in the precapillary branches. At the end of 24 hours many of the cotton

fibers were found in apposition to the endothelium and without any reaction about them. In other instances, large mononuclear cells were densely packed about the cotton fibers, and about some of these vessels were collections of similar cells (Fig. 2). These large mononuclear cells completely filled the lumina of the precapillary branches. The elastica was stretched and often ruptured at one point, permitting the mononuclear cells within the lumen to stream through and merge with any cells that had collected about the vessel. No multinucleated cells were present nor was there any thrombus formation. Forty-eight hours after the injection, most of the cotton fibers were surrounded by large mononuclear cells, among which multinucleated giant cells had made their appearance (Fig. 3). These granulomata often distended the lumina of the precapillary branches of the artery and arterioles to many times the original diameter. The elastica was stretched and in the smallest branches was ruptured, permitting the cell mass to move outward (Fig. 4). The surrounding tissue often appeared compressed and pushed aside by the cells as they passed outward from the lumen. Occasional mitotic figures were seen in the large mononuclear cells of the granuloma.

In the animals sacrificed during the first and second weeks after injection, the alterations had progressed further. Cotton fibers lying in the granulomata were carried outward as the cell mass moved through the gap in the elastica and came to lie well beyond the original limits of the vessel wall. At other times, when the elastica ruptured immediately beneath the particle of cotton, the cotton was carried outward at the rounded apex of the granulomatous mass of cells as they pushed from the lumen into the surrounding tissues. This was seen especially well in the precapillary branches. As the cell mass moved outward, a lumen began to appear again in the vessel. Sometimes the granuloma was being covered over by endothelial cells.

Cotton fibers, which had lodged in the portions of the arterial tree where the vessels had thick muscular walls, provoked a granuloma in some instances. At other locations the cotton was being covered by a few endothelial cells. Granulomata occasionally were seen in some of the large branches of the artery, as in an animal sacrificed at the end of 11 days. They partially filled the lumen and were covered by endothelial cells (Fig. 5).

As early as 11 days, the Wilder azocarmine-aniline blue preparations demonstrated numerous fibrils in the granulomata; some of these were argyrophilic, others were stained blue. The argyrophilic fibrils were more abundant in those granulomata that contained mainly large mono-

nuclear cells, and were much less numerous in those composed predominantly of multinucleated giant cells.

Sometimes a cotton fiber became lodged in a muscular artery in such fashion that one end was pointed directly against the wall; in those instances it could be readily seen that the fiber was beginning to penetrate the wall of the vessel.

As early as 4 weeks after injection, granulomata were found that lay entirely outside arterioles and small arteries. The opening through which the granuloma passed to the perivascular tissue had been filled with fibrous tissue in which, in many instances, delicate new elastic fibrils had been laid down. The inner surface of this new tissue closing the gap was covered with endothelium. The lumen of the vessel again was patent (Fig. 6). The passage of the granuloma through the vessel wall was not accompanied by hemorrhage.

Some granulomata were partly within the lumen and partly outside in the surrounding tissue (Figs. 7 and 8). The granuloma then assumed a shape somewhat like that of a dumbbell, with the narrow portion filling the opening in the wall of the arteriole or small artery. In this circumstance, the surface of the portion of the granuloma within the lumen was covered with endothelium. As the granuloma reached the tissue outside the vessel, the cells composing it were less densely packed; it appeared to have expanded. Serial sections often disclosed that granulomata apparently lying outside the vessel still had a small portion plugging a gap in the wall. Also it was obvious that the opening originally had been larger and that after the major portion of the granuloma had passed through, the gap in the wall was being filled in with new fibrous tissue (Figs. 9 and 10).

When the granuloma had formed in a larger artery, it was clearly seen that the cotton fiber was slowly penetrating the wall, often accompanied by a giant cell. Although the elastica interna had ruptured, the granuloma had not yet begun to move outward from the lumen.

Twenty-four animals were allowed to live from 2 to 6 months after injection. Cotton fibers were found in arteries indenting the wall and pushing the elastica interna outward; sometimes these fibers were incompletely surrounded by giant cells, in other instances they had been covered by connective tissue and endothelium. Other cotton fibers near the periphery of the lung were in the alveolar septa, bulging the septal wall inward into the alveolar space, or a pointed end of a fiber projected into the alveolus; a few were found in alveolar spaces, lying within giant cells that also contained anthracotic pigment (Fig. 11). This was

seen as early as 7 weeks after the injection. It was not possible to identify accurately the vessel in which the fiber had lodged because the lumen was reopened and the wall reconstituted after the fiber had escaped.

Numerous granulomata had passed from vessels into the adjacent tissue. As in the animals that were sacrificed in less than 2 months, these granulomata were composed of loosely arranged giant cells. Among them were occasional giant cells holding anthracotic pigment. A few vessels were found in which granulomata had remained in the lumina. These granulomata were reduced chiefly to compact masses of connective tissue which were covered by endothelium and incompletely or completely filled the lumina. Other granulomata had not yet entirely penetrated the vessel wall; a small portion of the cell mass still filled the narrow gap through which the major portion of the granuloma had passed.

Some examples were noted in which a granuloma lay near two arterioles. Each arteriole had a repaired defect in its wall adjacent to the granuloma that appeared to be too large to have been formed in a vessel of that size. It seems reasonable to assume that in these circumstances a small granuloma moved outward from each arteriole and by chance merged to form a single large one.

Restoration of the walls of the vessel, at the gaps through which granulomata had moved to the adjacent tissue or were in the process of escaping, was found in various stages regardless of the length of time the animals were allowed to live (2 to 6 months). There was a growth of connective tissue at the margin of the gap in the vessel wall. This new connective tissue seemingly formed a close-fitting collar about the granuloma as it passed outward; when the last trailing attenuated portion of the granuloma emerged, the gap was closed by connective tissue. Prior to this, endothelium had covered the surface of the hindmost part of the granuloma before it had entered the gap in the vessel wall. Thus the defect was repaired and no blood escaped from the lumen (Figs. 9 and 10).

As early as 2 months after injection certain cells, which had a distinctive appearance and staining reaction, were noted in the connective tissue filling the defect in the vessel wall. These cells resembled in all respects the smooth muscle cells of the media of the vessel (Fig. 12). Mitotic figures have not been found among the muscle cells of the media at the margin of the defect.

Some of the scars in the vessel walls were devoid of elastic tissue;



others contained delicate elastic fibrils (Fig. 6). In yet other vessels, an elastic tissue strand, as heavy as the original elastica interna, had been laid down and often joined the original elastica at a distance from the outwardly turned ends at the site of the rupture in the vessel wall (Fig. 12).

Granulomata occasionally were found in the largest branches of the artery within the lung. They were closely adherent to the intima over a considerable area and were covered with endothelium. In these vessels the granulomatous mass remained within the lumen and underwent organization.

Never did the partial or complete occlusion of the lumen of any of the branches of the pulmonary arteries lead to infarction of the lung.

### DISCUSSION

As might have been anticipated, the introduction of cotton fibers through a vein produced foreign body granulomata in branches of the pulmonary arteries. At first, the cells that collected about the particles of cotton resembled in every way large mononuclear wandering cells. These cells most probably were derived from the large mononuclear cells of the blood. Mitotic figures were seen in some of these collections of cells, indicating that cell proliferation was in progress within the granuloma. Very quickly, there appeared multinucleated giant cells among the cells collected about the cotton. The formation of the giant cells was probably similar to that described by Lewis,<sup>1</sup> following the inoculation of tubercle bacilli into hanging-drop preparations of blood.

The granulomata in the precapillary branches of the artery filled and distended the lumen of the vessel. In these branches the granulomata appeared to be under pressure; the elastica was stretched and ruptured within 24 hours. The granuloma then seemed to spring sharply outward into the surrounding tissue and carried with it the cotton fiber or fibers.

Granulomata in the arterioles and medium-sized arteries also distended the lumina of these vessels. The elastica interna was stretched and finally ruptured. It was very obvious that the rupture of the wall of the medium-sized artery occurred in that portion where the media had abruptly become thinner (Figs. 7 to 10).

As to the manner in which the granulomata escape from the vessels, there are two possibilities. The first of these is that the media and adventitia tear following the rupture of the internal elastica; the other possibility is that the cotton fiber penetrates the wall and gradually passes through, the cells of the granuloma following in the wake of the cotton

fiber. Both of these methods occurred. Granulomata were found emerging through a gap in the vessel wall, and the only cotton that could be discovered in serial sections was in the center of the granuloma. Other lesions were seen in which the cotton was penetrating the vessel wall, and cells comprising the granuloma were following it.

Regardless of the manner in which the vessel wall is damaged, once the gap in the wall has been formed, the granuloma moves outward into the surrounding tissue. One can only surmise what forces the granuloma outward. In the precapillary branches the mass of cells is so crowded together that the lumen is distended, and when the elastica is ruptured, the cells adjacent to the gap emerge through it. The gap, furthermore, is enlarged by the retraction of the elastica when it ruptures.

The passage of the granuloma through the walls of the arteries and arterioles with their muscular media is not so simple as in the precapillary branches. The arteries of medium size and the arterioles are distended by the granulomata. Once a defect is made either by tearing or penetration of the wall by the cotton particles, the cells of the granuloma move into the gap. The granulomata are under the constant pressure of the blood in the pulmonary vascular tree, and, in addition, the heightened pressure during systole probably stretches the hole in the wall and forces the forward portion of the granuloma further into the defect, thus widening and deepening it. When the defect involves all layers of the vessel, the granuloma begins to emerge to the outside and pushes ahead of it the surrounding loose tissue. With each cardiac systole more of the granuloma is forced outward until all of it has emerged from the vessel. Probably the blood pressure within the precapillary branches also plays a part in the passage outward of the granulomata. It is not clear what part the movement of the lung during respiration may play in the process.

Reparative processes begin quickly with the proliferation of fibroblasts. These form a collar about the column of cells of the granuloma and appear to fit snugly about it. Endothelial cells cover the surface of the granuloma and separate it from the circulating blood. When the last portion of the granuloma passes through the intima, the continuity of endothelial surface has been restored. As rapidly as the last part of the granuloma penetrates the media, connective tissue cells fill the gap behind it. When the granuloma finally has passed entirely through the wall, the defect has been filled with connective tissue. It would thus appear that the emergence of the granuloma to the tissues outside the vessel is comparatively slow and affords ample time for restoration of the wall.

The granuloma, or portion of it lying outside the vessel, has a much

less compact structure. The giant cells are more loosely arranged and the cell mass occupies a greater space than it did in the lumen of the vessel. This is very likely due to the fact that it is now no longer confined to a restricted space in the lumen of the vessel nor subjected to the pressure of the blood within the vessel. Also, additional giant cells are formed from the tissue wandering cells that are attracted by the presence of the foreign body. That some of the cells come from the surrounding tissue is evidenced by the anthracotic pigment to be seen in an occasional giant cell; similar pigment has not been found in any cell in a granuloma within the lumen of a vessel except when rupture of the vessel wall has occurred. The presence of anthracotic pigment within the cells in the lumen is a rare occurrence (Fig. 13).

The movement of the cotton fibers does not cease when the granuloma reaches the tissues around the vessel. Long, slender, pointed particles of cotton are to be seen pushing inward the wall of a nearby alveolus, or the naked end of such a fiber projects into the alveolar space. Moreover, tiny fragments of cotton that have escaped through the wall of a pre-capillary branch of the artery ultimately are found within a giant cell in an alveolus. Probably the constant expansion and contraction of the lung is responsible for the movement of the cotton fibers in the tissue after they have escaped from the vessels.

The compact granuloma within the lumen of the vessel, even in its earliest stages, is impervious to the entrance of blood. Red blood cells and hemosiderin are not found within it. That there is no hemorrhage as the granuloma passes from the vessel is due to the fact that the cell mass acts as a tightly fitting stopper in the defect in the vessel wall. Furthermore, the quick covering of the granuloma with endothelial cells shuts it off from contact with the blood. In addition, the passage of the granuloma to the outside tissues is seemingly slow and reparative processes have sufficient time to keep the margins of the gap tightly closed about the emerging granuloma and to fill the space behind it. After the granulomata have escaped from the vessels, the lumina are again patent.

The restoration of the continuity of the wall of the vessel is complete as soon as the granuloma has passed through it. At first the defect is filled with connective tissue covered with endothelium. Soon delicate elastic fibrils are noted in this scar tissue. Later a more compact strand of elastic tissue is found either incompletely or entirely spanning the area filled with scar tissue. Often this new elastica joins the original ruptured

elastica interna at a distance from its outwardly turned ends. Further changes take place in the scar that fills the defect in the vessel wall. As early as 2 months after injection of the cotton, plump cells having the morphologic and tinctorial properties of smooth muscle cells are distinguishable among the connective tissue elements of the scar. It is not likely that these come from the smooth muscle of the media for no evidences of proliferation of this muscle are to be found. The formation of muscle is most probably due to metaplasia of fibroblasts and is quite analogous to the familiar formation of smooth muscle about the vessels in an organized, canalized thrombus.

The collateral circulation was adequate to prevent infarction of the lungs in the experimental animals; in those instances in which cotton fibers were found in human pulmonary arteries, there was only very slight reduction of the lumina and no infarcts had resulted.

The lack of thrombus formation about the cotton fibers when they lodge within the lumen of the vessel is a striking feature, true in both the experimentally injected animals and in the human cases.

No report of cotton fibers in the pulmonary arteries of the human nor of any experiments in which cotton fibers have been experimentally injected into the venous circulation of animals could be found in the literature. Flory<sup>2</sup> reported the formation of granulomata about cholesterol injected into the pulmonary arterial circulation of rabbits. His experiments were of short duration and no description was given of any movement of the granulomata through the walls of the arteries.

#### SUMMARY

Cotton fibers accidentally introduced into the pulmonary arterial circulation of the human provoke a foreign body reaction. When cotton fibers are injected into the iliac veins of rats and lodge in the pulmonary arteries, foreign body granulomata are formed. These granulomata in the larger branches of the arteries undergo organization. In arteries of medium size, arterioles, and precapillary branches, the granulomata distend the lumina and often escape through defects in the walls. The defect in the vessel wall is produced by penetration of the cotton fiber through it and also by actual tearing of the wall. The gap is at first filled with fibrous tissue; later some smooth muscle and frequently a new elastica interna are formed. The scar is covered by endothelium. The lumen is again patent after the granuloma has escaped. Some of the cotton fibers ultimately come to lie in alveolar spaces and are surrounded

by foreign body giant cells. There is no thrombosis nor hemorrhage associated with these granulomata, nor does the process lead to infarction of the lungs.

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#### DESCRIPTION OF PLATES

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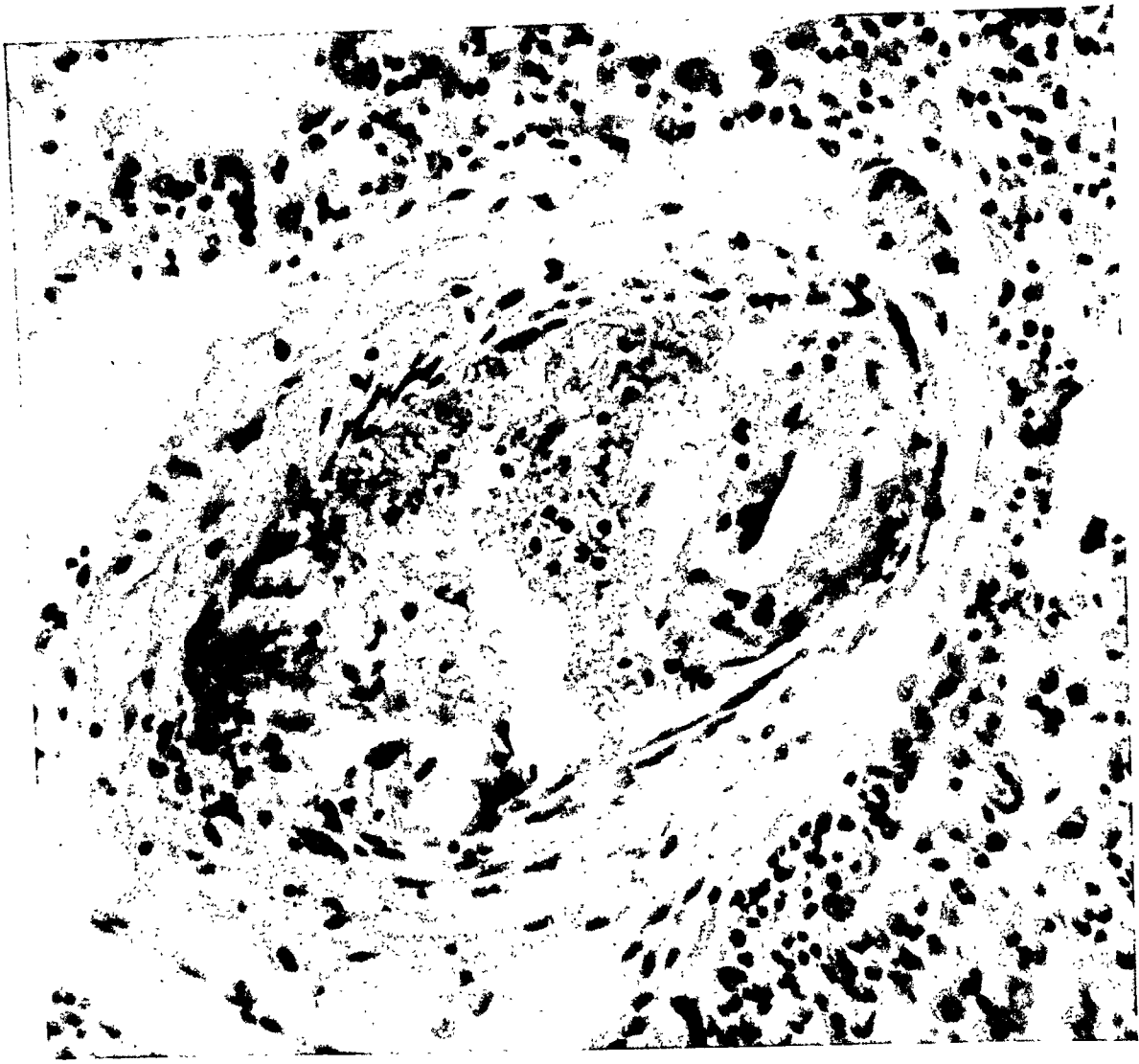
The cotton fibers do not stain. In some of the photomicrographs they show faintly in the granulomata; in others, seemingly empty spaces in the granulomata indicate the size and location of the cotton fibers.

#### PLATE 86

FIG. 1. Cotton fibers in a pulmonary artery of a child, 14 months of age, who had been given a blood transfusion. The cotton is surrounded by giant cells. Hematoxylin and eosin stain.  $\times 390$ .

FIG. 2. Rat, 24 hours after injection. Cotton fiber in a precapillary branch of a pulmonary artery, surrounded by large mononuclear cells. The vessel is distended, the elastica has ruptured. Hematoxylin and eosin and Weigert's elastic tissue stains.  $\times 660$ .

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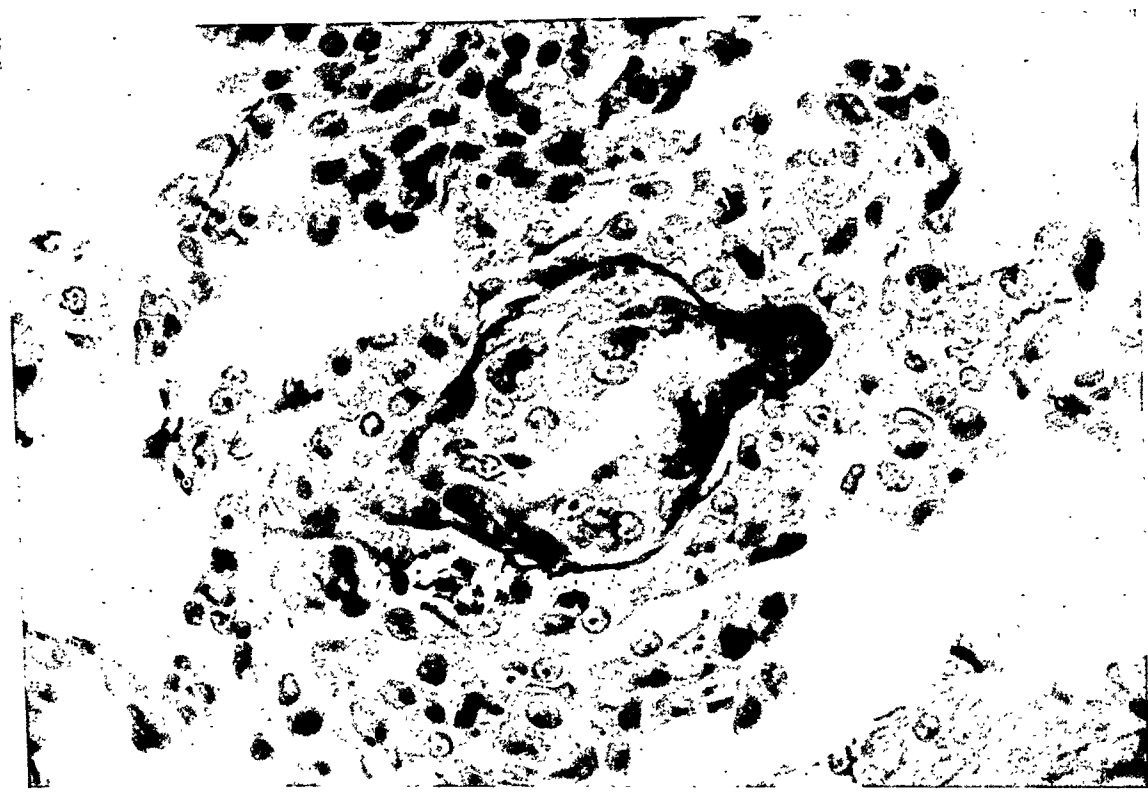
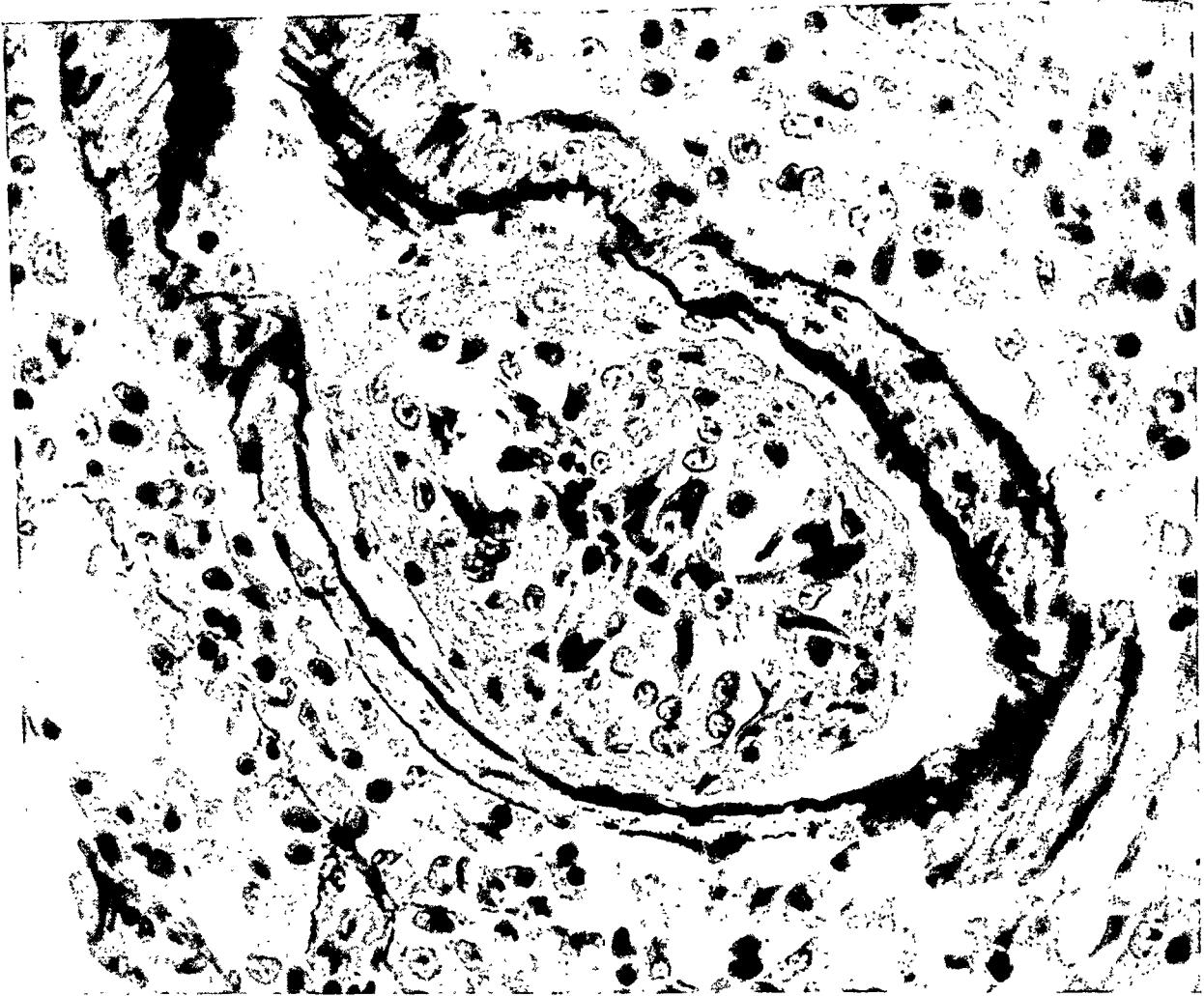


PLATE 87

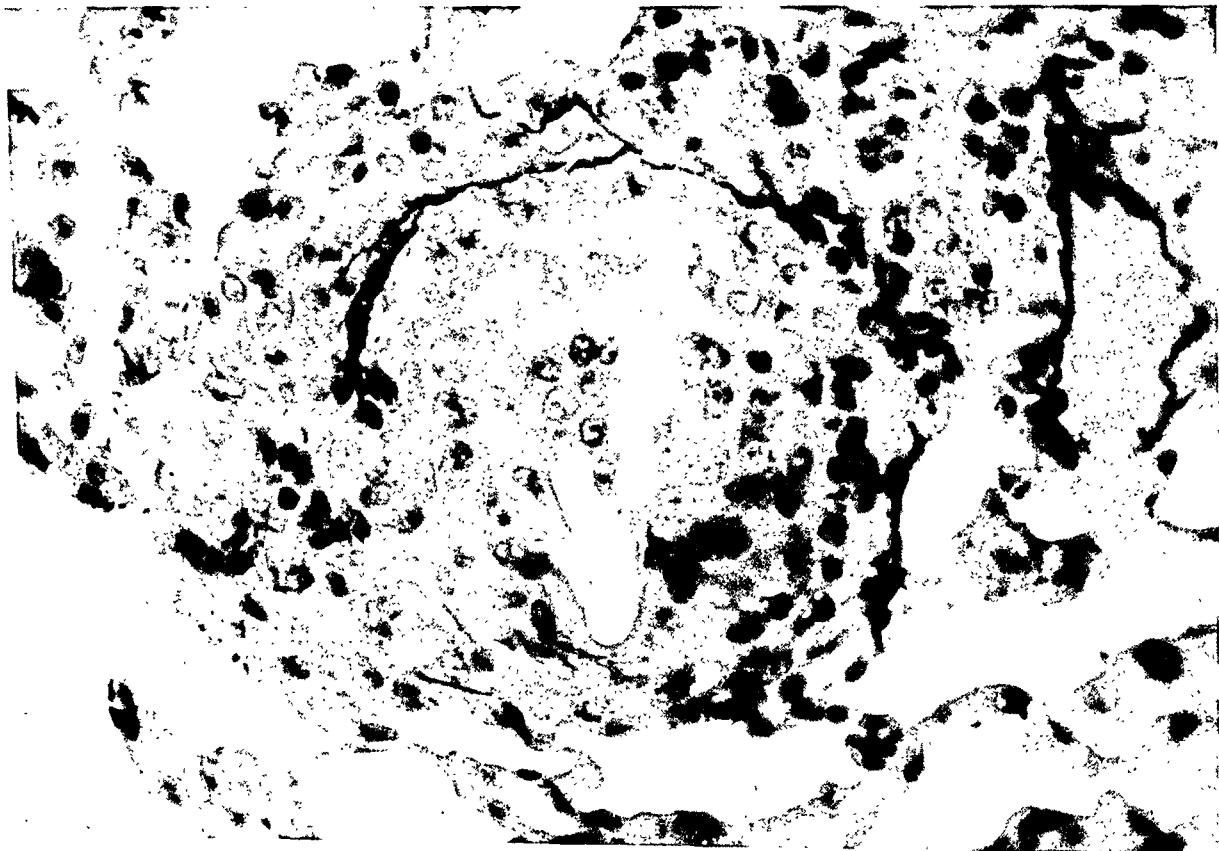
FIG. 3. Rat, 48 hours after injection. Granuloma in a pulmonary artery of medium size. Cotton is seen near the center of the granuloma. The lumen is distended. Hematoxylin and eosin and Weigert's elastic tissue stains.  $\times 660$ .

FIG. 4. Rat, 48 hours after injection. Granuloma in precapillary branch of pulmonary artery. The granuloma has distended the lumen and is passing outward through a wide gap in the vessel wall, where the elastica has ruptured. A cotton fiber is near the center of the granuloma. Hematoxylin and eosin and Weigert's elastic tissue stains.  $\times 660$ .

3



4



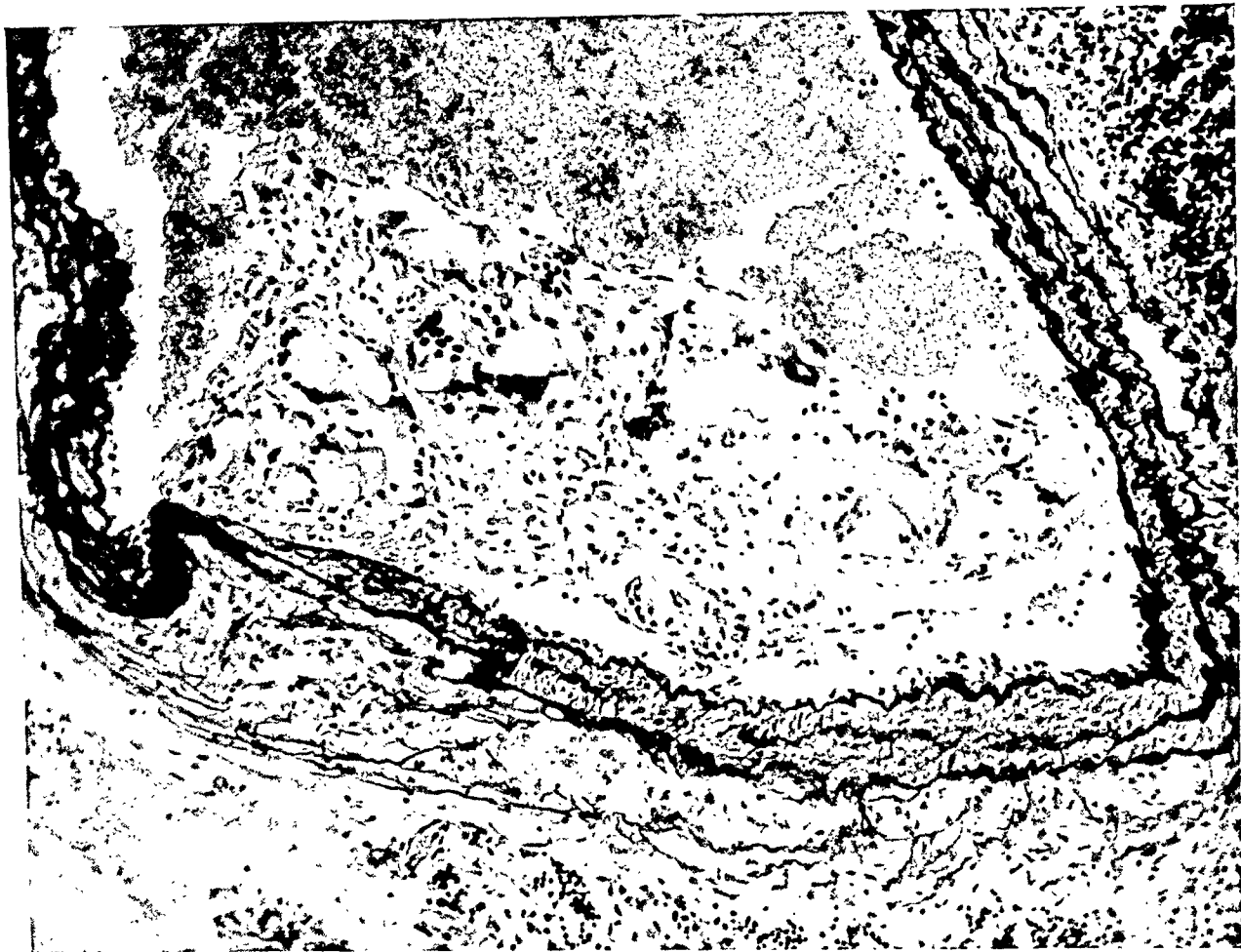


## PLATE 88

FIG. 5. Rat, 11 days after injection. Granuloma with many fragments of cotton in large branch of pulmonary artery. The surface of the granuloma is covered by endothelial cells. Hematoxylin and eosin and Weigert's elastic tissue stains.  $\times 190$ .

FIG. 6. Rat, 4 weeks after injection. Granuloma lying entirely outside medium-sized branch of pulmonary artery. The defect in the vessel wall is closed by fibrous tissue in which are delicate elastic fibrils. Lumen is again patent. Hematoxylin and eosin and Weigert's elastic tissue stains.  $\times 390$ .

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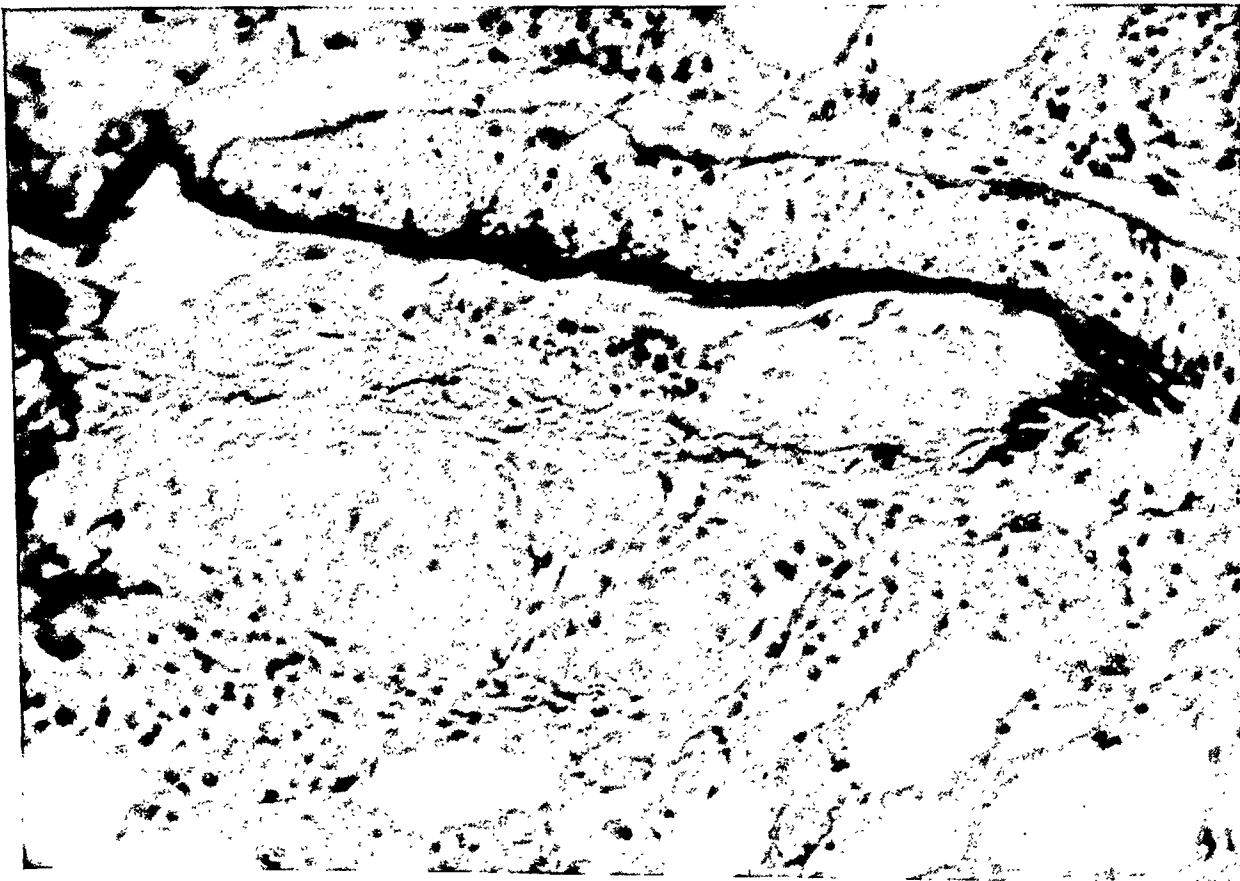
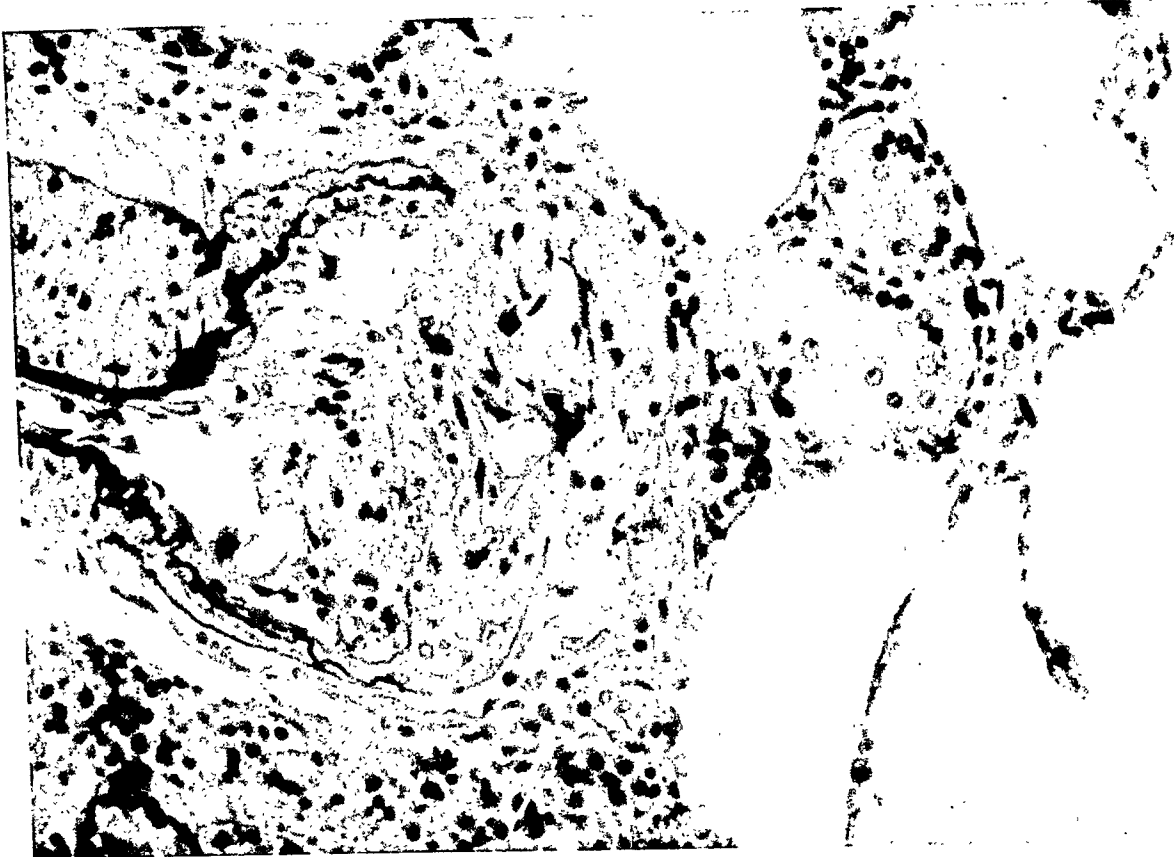


PLATE 89

FIGS. 7 and 8. Rat, 11 days after injection. Granulomata in medium-sized branches of pulmonary artery. The granulomata are passing outward through defects in walls of vessels. In Figure 7 it is to be noted that rupture of the vessel occurred in a normally thinner portion of wall. Hematoxylin and eosin and Weigert's elastic tissue stains.  $\times 390$ .

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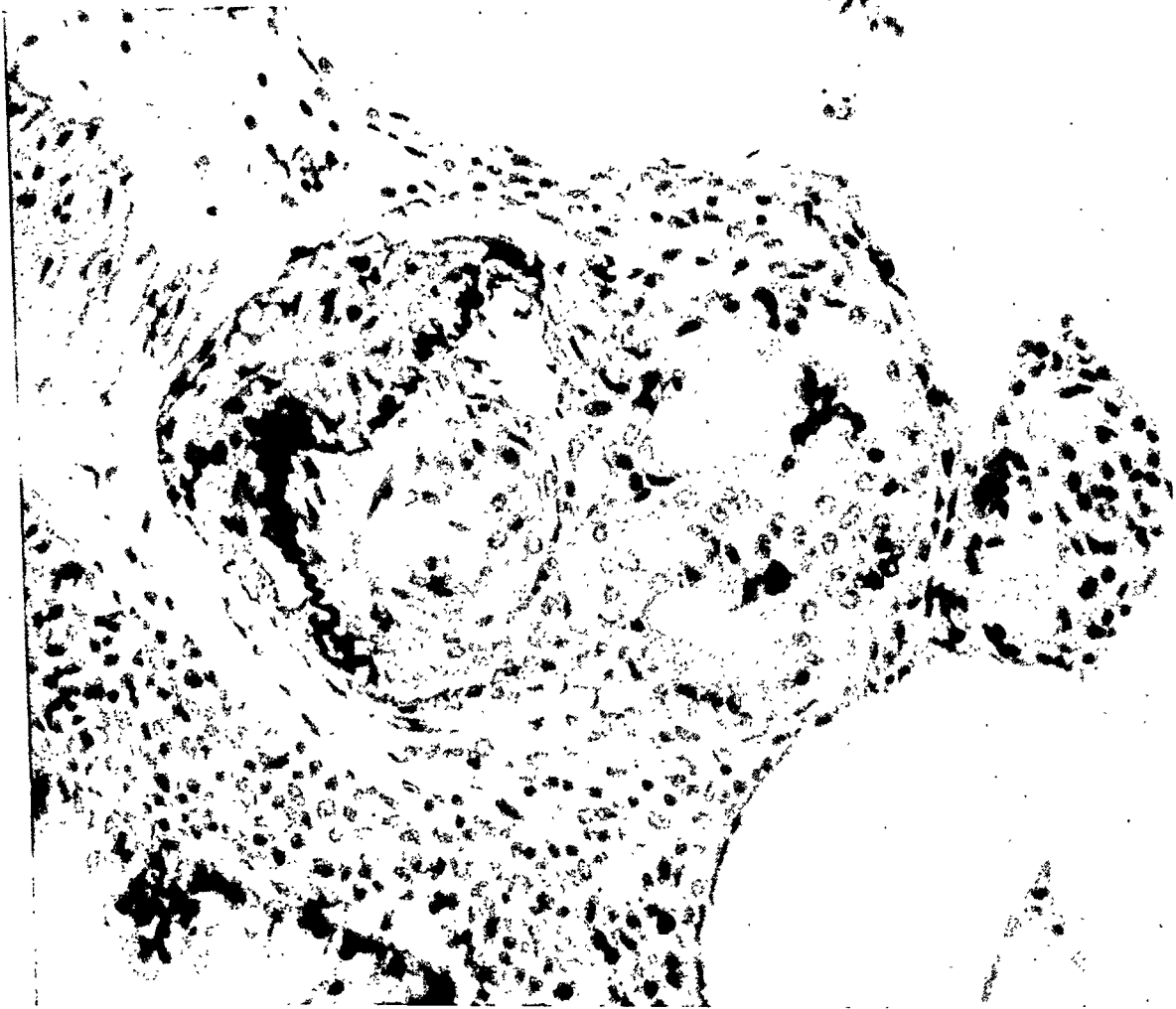


PLATE 90

FIGS. 9 and 10. Rat, 2 months after injection. Medium-sized branch of pulmonary artery. In Figure 9 the granuloma appears to be entirely outside the vessel and the defect in the wall closed completely by fibrous tissue. Figure 10 is from another of the serial sections through the granuloma. The last portion of the granuloma has just emerged from lumen and is covered by endothelium. The rupture occurred in the normally thinner portion of the vessel wall. Hematoxylin and eosin and Weigert's elastic tissue stains.  $\times 280$ .

9



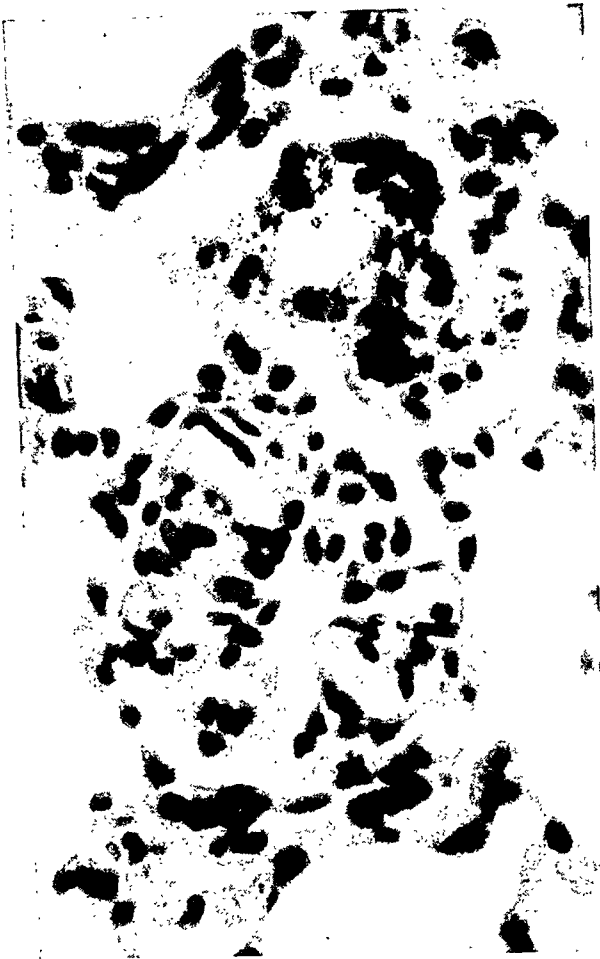
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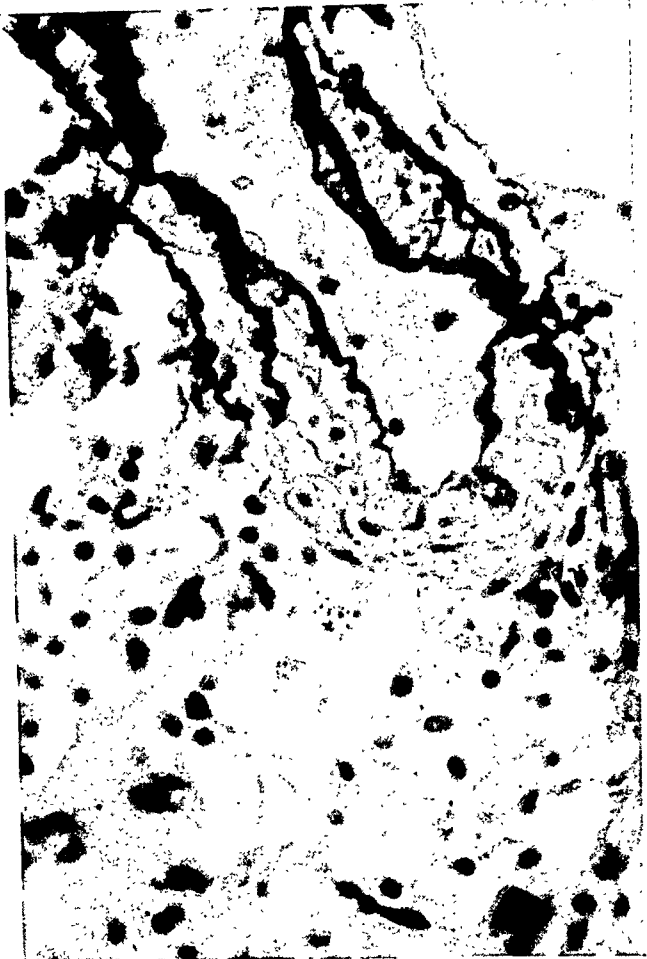
## PLATE 91

- FIG. 11. Rat, 2 months after injection. A small piece of cotton in an alveolus. The cotton is surrounded by a giant cell containing anthracotic pigment. In a nearby septum there are two pieces of cotton, each with a giant cell, near two precapillary branches of an artery. Hematoxylin and eosin stain.  $\times 588$ .
- FIG. 12. Rat, 3 months after injection. Granuloma lying entirely outside medium-sized artery. A defect in the wall of the artery is filled by fibrous tissue in which are cells identical with smooth muscle. New elastic tissue joins the ruptured original elastica at a distance from the outwardly turned ends. Rupture occurred through a normally thinner portion of vessel wall. Hematoxylin and eosin and Weigert's elastic tissue stains.  $\times 588$ .
- FIG. 13. Rat, 8 days after injection. Granuloma in a precapillary branch of artery. The lumen is greatly distended. The elastica has ruptured in two places and the granuloma is escaping through a wide gap in the vessel wall. Anthracotic pigment occurs in two of the giant cells. Hematoxylin and eosin and Weigert's elastic tissue stains.  $\times 588$ .

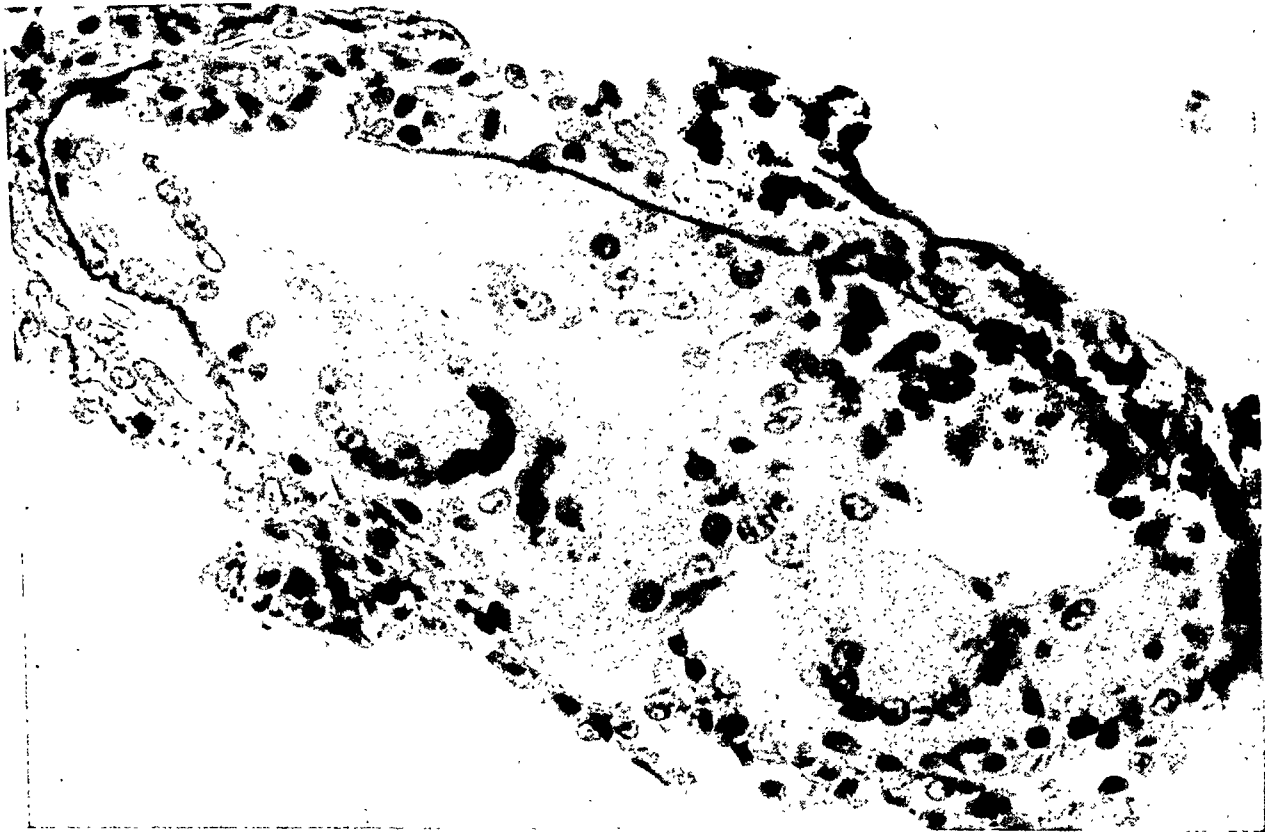
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13







# HISTOCHEMICAL STUDIES IN GAUCHER'S DISEASE\*

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In Gaucher's disease there are many large foamy cells in the spleen and liver, and often in the bone marrow and lymph nodes.<sup>1,2</sup> Most pathologists now agree that these cells arise from the reticulum and from histiocytes in the spleen, liver, lymph nodes, and bone marrow.<sup>1,2</sup> Several investigators have shown that kerasin, a cerebroside, is accumulated in Gaucher's disease.<sup>3-6</sup>

The use of histochemical methods to identify and localize kerasin in sections of spleens from patients with Gaucher's disease seemed worthy of trial. The physical properties of kerasin are of importance in such an approach. It is soluble in warm (65° C.) methyl alcohol and chloroform (1:1), in warm (65° C.) ethyl alcohol, and in pyridine. It is insoluble in cold methyl alcohol, ethyl alcohol, xylene, ether, petroleum ether, and acetone. An ultraviolet absorption peak for kerasin has been reported at 2675 to 2700 Angstrom units.<sup>7</sup>

Kerasin is composed of lignoceric acid, sphingosine, and a hexose, usually galactose,<sup>3</sup> but sometimes glucose. With a compound of this nature the periodic acid-leukofuchsin method<sup>8,9</sup> might be expected to produce a positive reaction. In that case one might expect the hexose to be oxidized by the periodic acid to a diglycollic aldehyde. When this reacts with the fuchsin-sulfurous acid reagent, it forms the corresponding colored (rose-purple) addition product. This was confirmed by means of a spot test with pure kerasin isolated from human brain. In sections the altered carbohydrate remains bound to the insoluble components of kerasin and enables the colored product to be accurately localized microscopically.

## METHODS AND OBSERVATIONS

Spleens† removed from 3 patients with Gaucher's disease were available for study. A block from each spleen was fixed in 10 per cent formalin. A second block was taken from one fresh specimen and fixed by freezing and drying. Paraffin sections from all four blocks were passed through xylene, absolute alcohol, and 70 per cent alcohol at room temperature. The sections were then treated for reactive carbohydrate groups by Hotchkiss'<sup>9</sup> method.

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† Drs. Paul Klemperer and Otto Saphir each very kindly furnished material from one of his cases of Gaucher's disease.

On microscopic examination a brilliant rose-purple color was seen in the Gaucher cells (Figs. 1 and 2). The results seemed equally good with formalin-fixed or frozen-dried material. The red pulp (Fig. 1) was mostly replaced by irregularly shaped spaces containing many large, oval, rose-purple Gaucher cells. The reticulum fibers of the spleen stood out boldly. No rose-purple cells were seen in the centers of the lymphoid follicles, but toward the periphery a few small ones were noted (Fig. 2). Surrounding the lymphoid follicles were masses of brilliantly colored Gaucher cells. These showed the characteristic eccentric position of the nucleus which itself was free of the colored compound. The cytoplasm was packed with granules of rose-purple material.

An attempt was made to extract the reactive material from the cells using solvents known to dissolve kerosin. Sections from the four blocks were deparaffinized and successively immersed in warm (65° C.) methyl alcohol-chloroform mixture (1:1) for 12 hours and warm ethyl alcohol for 12 hours. They were then stained as before. The Gaucher cells showed as vivid a color reaction as in the unextracted sections. The question then arose whether kerosin can be extracted from sections after fixation with formalin or by freezing and drying.

Accordingly, an experiment was arranged in which one white rat was given intravenously 25 mg. of purified kerosin prepared from human brains and each of 4 rats was given 100 mg. intraperitoneally. Autopsies were performed after 20 minutes, and after 4, 8, 24, and 72 hours, respectively. Two blocks were taken from the liver of each animal; one was fixed in 10 per cent formalin and the other by freezing and drying. Paraffin sections from all blocks were treated by the periodic acid-leukofuchsin method, avoiding the use of hot solvents. Many uniformly rose-purple, discrete, highly refractile globules about 1 or 2  $\mu$  in diameter were seen in the cytoplasm of the Kupffer cells. These globules were thought to be kerosin. Their specific location in Kupffer cells distinguished them from glycoprotein granules seen in the cytoplasm of many different cells. Glycoprotein granules were not refractile, varied much in size, took the rose-purple color with varying intensity, and had irregular fuzzy outlines.

Extraction of the material believed to be kerosin was undertaken. Deparaffinized sections from the same blocks of rats' livers were placed in warm (65° C.) methyl alcohol and chloroform solution for 12 hours. After extraction they were treated by the periodic acid-leukofuchsin method. Examination disclosed a marked decrease in the number of globules (presumably kerosin) as well as a marked reduction in the

number of Kupffer cells containing globules. The glycoprotein granules in these sections remained unaltered. The observed differences in extracted and unextracted sections were less marked in the 2 animals killed 24 and 72 hours after injection than in the other animals. Sections of liver from a control animal showed no refractile globules in the Kupffer cells. Exposure of deparaffinized sections to a warm (65° C.) methyl alcohol and chloroform mixture produced no detectable histologic alterations.

A formalin-fixed deparaffinized section from each of the 3 Gaucher's spleens and a frozen-dried deparaffinized section from one fresh specimen were examined with the ultraviolet microscope. The Gaucher cells in all sections almost completely absorbed ultraviolet light at 2800 Angstrom units. On the other hand, there was no peak absorption band when purified kerasin was exposed to ultraviolet light.

A small amount of formalin-fixed material from the spleen of a patient with Niemann-Pick's disease was available for study. Deparaffinized formalin-fixed sections were treated by the periodic acid-leukofuchsin method. None of the vast numbers of typical foam cells in the pulp showed the rose-purple color.

### DISCUSSION

As anticipated, purified kerasin gives a rose-purple color when subjected to the periodic acid-leukofuchsin method. The galactose in the kerasin probably provides the adjacent hydroxyl groups requisite for oxidation to aldehyde by periodic acid.

The appearance of the rose-purple color in the sections from human Gaucher spleens demonstrates that kerasin may be present and localized within the Gaucher cells. Heretofore the identification of kerasin has been by macrochemical extraction from large portions of spleen. The inference was then made that the abnormal substance (kerasin) was present in the multitudes of foam cells seen microscopically. Our evidence is the most specific yet advanced to show that kerasin occurs within the Gaucher cell.

Kerasin apparently was not extracted from the sections of Gaucher's spleens exposed to a mixture of warm methyl alcohol and chloroform. When kerasin is injected into rats it appears in the Kupffer cells of the liver as particulate material. With these solvents it can be removed readily from sections of frozen-dried or formalin-fixed tissue. Decreased amounts were extracted from tissues of animals living 24 and 72 hours. A possible explanation is that metabolic processes might have fixed the

splenic kersin in place by uniting it with another substance. The idea that protein and cerebroside might be linked is attractive. Such a linkage, if demonstrated, might explain the failure to extract kersin with the appropriate solvents. On the other hand, there is little chemical evidence for the existence of such a lipid-protein complex.

Ultraviolet absorption studies support the idea that a protein is present in the Gaucher cell. Dworacek and Pesta<sup>7</sup> reported that maximum absorption for kersin occurred between 2675 to 2700 Angstrom units. Using purified kersin, it was not possible to confirm their findings; *i.e.*, in the range 2300 to 3300 Angstrom units, kersin was found to have no region of maximal absorption.

Under the ultraviolet microscope, Gaucher cells showed a strong absorption band at 2800 Angstrom units. This indicates the presence of large amounts of protein in Gaucher cells, for it is well known that proteins absorb ultraviolet light of 2800 Angstrom units. It is possible, but not proved, that this protein and kersin are joined together.

Figure 1 shows the striking reaction of the reticulum to the periodic acid-leukofuchsin method. This is due to the high content of reactive material in the reticulum fibers. The polysaccharide bound to the protein reacts in the same manner as does the kersin. The color of the glycoprotein granules within the cells in rats' livers can be explained on the same basis.

The observation, illustrated in Figure 2, that the cells contain more rose-purple material as they approach the periphery of the lymphoid follicle is of interest. It is possible that here both lymphocytes and Gaucher cells are seen arising from the primitive cells, offspring of the reticulum of the lymphoid follicle. This can be regarded as further evidence that Gaucher cells arise from the reticulum. It also strengthens the belief that in Gaucher's disease the fault lies within the cell involved, that is, the Gaucher cell is not a phagocyte collecting kersin from the passing stream. It is probable that as the cell matures, the inherent metabolic error becomes apparent.

The material from the case of Niemann-Pick's disease illustrated the differential diagnostic possibilities of the periodic acid-leukofuchsin method. The pathognomonic lipid in this disease is sphingomyelin.<sup>2,3</sup> Its molecule does not include a hexose. In agreement with this, the rose-purple color failed to appear when the method was tried on pure sphingomyelin and on tissue from Niemann-Pick's disease. The method would seem applicable to smears of material collected at sternal puncture in which the presence of large cells with abundant foamy purple-red cyto-

plasm would certainly bring Gaucher's disease into consideration. In this manner it is possible histologically to differentiate between Gaucher's disease and Niemann-Pick's disease.

### CONCLUSIONS

It has been demonstrated that kerasin, because of its hexose component, gives the characteristic rose-purple response with the periodic acid-leukofuchsin method.

For the first time kerasin has been localized within the Gaucher cell. Failure to extract kerasin from the cell suggests that it may be present in combination with some other substance.

The periodic acid-leukofuchsin method has differential diagnostic possibilities for the lipidic diseases. Whereas Gaucher cells develop an intense rose-purple, the characteristic foam cells of Niemann-Pick's disease remain colorless with this procedure.

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[ Illustrations follow ]

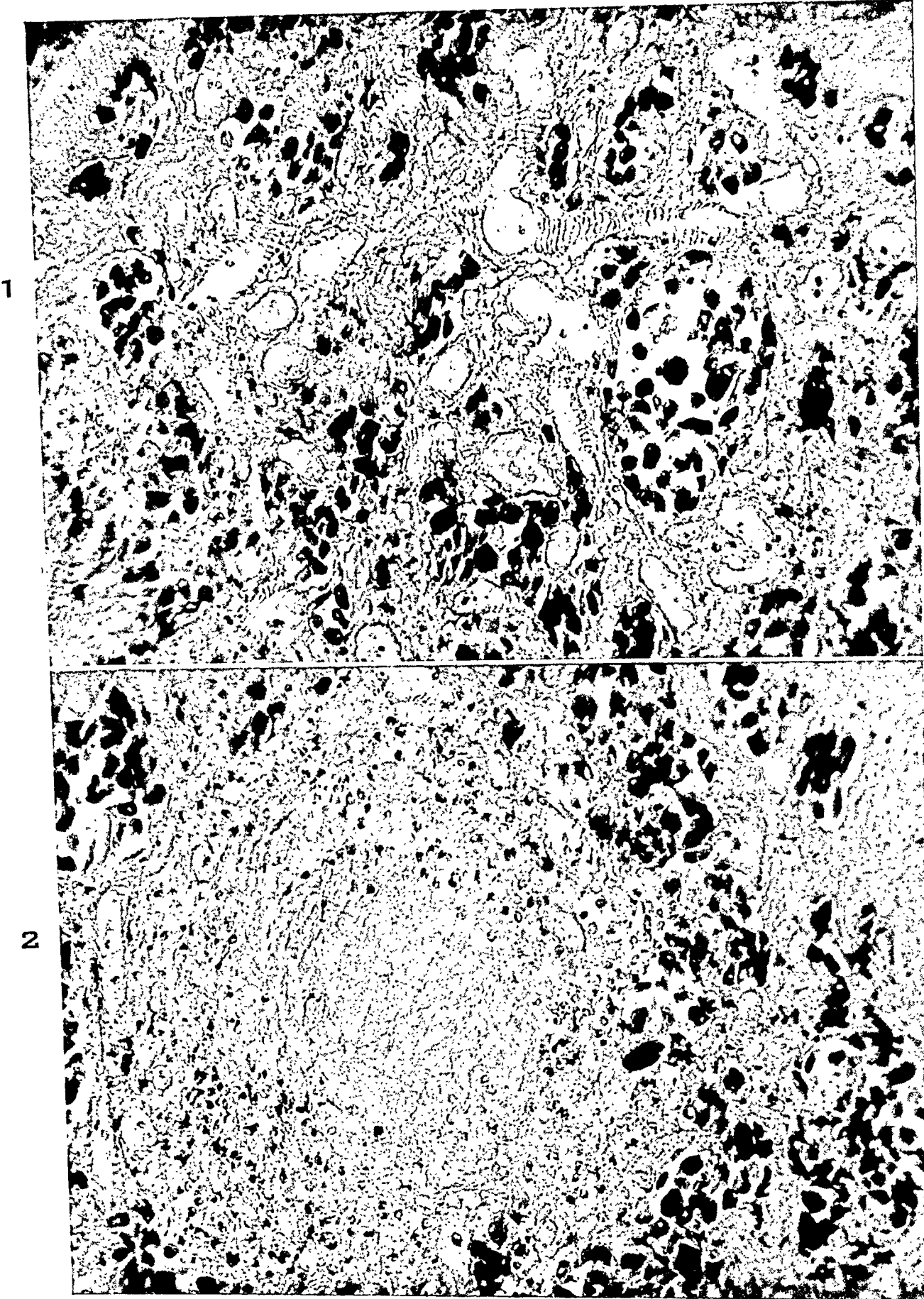
## DESCRIPTION OF PLATE

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### PLATE 92

FIG. 1. Spleen, Gaucher's disease. The pulp is mostly replaced by irregularly shaped spaces containing many large, oval, Gaucher cells. The reticulum is clearly shown. Formalin (10 per cent) fixation. Periodic acid-leukofuchsin method of staining.  $\times 240$ .

FIG. 2. Spleen, Gaucher's disease. The cells contain more periodic acid-leukofuchsin reacting material as they approach the periphery of the lymphoid follicle. Formalin (10 per cent) fixation. Periodic acid-leukofuchsin method of staining.  $\times 240$ .







# A TRANSPLANTABLE SPLENIC TUMOR RICH IN MAST CELLS OBSERVATIONS ON MAST CELLS IN VARIED NEOPLASMS\*

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Numerous investigators have noted a mast cell increment in and about many tumors.<sup>1,2</sup> Many recent studies have suggested a relation of mast cells to heparin formation.<sup>3-5</sup> Hyperheparinemia has been found to be the cause of certain hemorrhagic tendencies.<sup>6</sup>

The finding of a transplantable splenic tumor of mice, characteristically rich in mast cells, called our attention to problems related to these cells. Indeed, the question arose whether the cells of these splenic neoplasms themselves are related to mast cells. The predominant cell type of these splenic tumors<sup>7</sup> is a polygonal epithelium-like cell, thought to be a neoplastic derivative of splenic reticulum. It will hereafter be referred to as the ER (epithelium-like reticulum) cell. For the study already reported, special stains showing mast cells were not employed and the richness of these neoplasms in mast cells was overlooked. Subsequently, sections from the spontaneous neoplasms of this type previously reported have been restained for mast cells and it has been found that their presence in these ER tumors is characteristic.

Histologic studies were undertaken to determine the relation of these two types of cells, to find out how frequently and characteristically neoplasms of mice of this and other types contain mast cells, and to obtain information on the histogenesis of mast cells.

## MATERIAL AND METHODS

*Material.* Sections from the following neoplasms of mice, fixed in Zenker's formol solution, were stained with Giemsa's stain for mast cells: 18 spontaneous benign ER splenic tumors; 4 other splenic tumors; 6 transplanted ER splenic tumors; 7 miscellaneous spontaneous carcinomas; 12 miscellaneous sarcomas (3 of which were transplanted, 2 induced, and 7 spontaneous); 9 transplanted carcinomas; 44 spontaneous leukemias; 15 spontaneous pulmonary adenomas; 3 transplanted endotheliomas; and 162 varied transplanted ovarian tumors.

*Fixation.* Zenker's-formol mixture seemed adequate for fixation. Apparently the granules were as easily demonstrable in routine autopsies made up to 24 hours after death as in tissues fixed immediately after death. Thionin blue stains, made on several imprints and on a few

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sections, did not demonstrate the mast cell granules better than Giemsa's stain, and the latter gave a superior staining of the general background. Routine hematoxylin and eosin sections seldom disclosed mast cells.

*Transplantation.* Transplantation of these splenic tumors, reported here for the first time, was attempted by cutting up the tumor in Tyrode's solution and injecting or implanting the tumor fragments in the subcutaneous tissue or spleen, in the anterior chamber of the eye, or intravenously. Numerous animals were irradiated with approximately 400 r. either before or shortly after injection to increase their susceptibility to the growth, the site of injection being shielded. Because of the chronicity of the tumors the irradiation was repeated in several instances. The factors of irradiation were: 400 r.: 140 kv., 5 ma.; filter, 1 mm. Al; target distance, 28 cm.; time, 5 minutes, 38 seconds. 200 r.: 140 kv., 5 ma.; filter, 1 mm. Al; target distance, 28 cm.; time, 2 minutes, 49 seconds. 350 r.: 140 kv., 5 ma.; filter, 1 mm. Al; target distance, 28 cm.; time, 4 minutes, 58 seconds.

#### MORPHOLOGIC CHARACTERISTICS OF ER SPLENIC TUMORS

These spontaneous neoplasms usually were single and circumscribed but not encapsulated, ranging from 4 to 22 mm. in greatest diameter. They were gray-white, soft, and frequently spotted with areas of hemorrhage or necrosis.<sup>7</sup> Erythropoietic foci were frequent in these tumors (Fig. 1). The neoplastic cells were polygonal with finely granular, slightly acidophilic cytoplasm and a large vesicular nucleus containing one or two nucleoli (Figs. 1 and 5). The predominant cells were unlike the usual reticulum cells hitherto described in man and in animals. They are designated as "ER" cells only for expediency and in the belief that their most likely precursor is the reticular framework cell of the spleen.

In hematoxylin and eosin preparations numerous dark-staining polymorphous cells were noted in and about reticular cells. These resembled Kupffer cells, and when stained with Giemsa's stain were found to be loaded with granules as seen in Figure 2. All but one of the 18 spontaneous splenic tumors of this type which were examined contained numerous mast cells.

The size of the mast cells in the tumors varied from about 8 by 8  $\mu$  to about 11 by 30  $\mu$  with an average diameter of about 11.5  $\mu$ . The mast cells were in contact with tumor cells. Some were in the center of a group of tumor cells while others were at the periphery of such groups, with cytoplasmic projections extending between the tumor cells. At the periphery, some were located between the tumor cells and capillaries and only

rarely could they be seen in the capillaries. They also were in the "capsule" of the tumor and were abundant about the connective tissue stroma and about the adventitia of the vessels.

The shape of the mast cells was variable: round, oval, pear-shaped, elongated, or ramifying. The nuclei usually were oval, sometimes round. The nuclear-cytoplasmic ratio was approximately 1 to 1½. The chromatin of nuclei of mast cells usually was dense and took on a homogenous blue color with Giemsa's solution (Figs. 3 and 8). The nuclei of ER cells were larger, always nearly round; the chromatin structure was loose and there were one or two nucleoli.

The metachromatic granules of the mast cells were coarse, although finer than those of normal mast cells, and usually so dense that the nucleus was barely detectable. However, when the sections were understained, it was possible to see clearly the blue-colored nuclei amidst the violet-colored granules (Figs. 3 and 8). Rarely, an unstained zone could be seen immediately around the nucleus where the granules were lacking. Since no special precautions were taken to preserve the granules before fixation or during the process of staining, no special significance can be attached to differences in density of granules.

In general mast cells were not connected with one another. In some places they appeared to make contact by cytoplasmic extensions (Fig. 8) and they occurred frequently in groups. Mitotic figures were not noted among them but rarely binucleated forms occurred (Fig. 8). The latter were suggestive of amitotic division. Occasionally a spherical body with size, shape, and staining qualities of a red cell was seen in the cytoplasm of a mast cell; or a vacuole was noted in similar location.

The distribution of mast cells in spontaneous splenic tumors is summarized in Table I.

TABLE I  
*Distribution of Mast Cells in Spontaneous ER Splenic Tumors*

Mast cell distribution	No. of mice	Central parts	Peripheral parts	Connective tissue stroma	Peri-vascular area	Peri-hemorrhagic area
Uneven	10	13	17	19	17	18
Even	7	13	13	13	13	13

In the last five columns the figures indicate the number of mast cells per 0.3 sq. mm. area.

In 7 tumors mast cells were evenly distributed; in 10 the number varied in different parts of the tumor. The greatest density was noted in the peripheral region and about the heavy strands of connective tissue stroma traversing the tumor. In some parts of the tumor, notably about

hemorrhages, mast cells usually were abundant. They were rare in the splenic tissue surrounding the tumor and also rare in the normal portions of the spleen. In only one mouse with this type of tumor was the red pulp full of mast cells as described by Deringer and Dunn<sup>8</sup> in a study of mast-celled growths in mice.

Mouse Akh 152 died at 8 months of age and had a tumor 1.5 cm. in diameter in an otherwise normal spleen. The tumor was gray with brown-red areas of hemorrhage and necrosis. Its microscopic appearance was typical of an ER neoplasm with large numbers of mast cells. At the periphery of the tumor there were dense collections of mast cells. The pulp of the spleen contained many mast cells in circumscribed foci. Binucleated forms were numerous and chain formations infrequent. Mast cells in the spleen were somewhat larger (averaging about 14 by 11.6  $\mu$ ) than those in the tumor, averaging about 11 by 11  $\mu$ . Almost every mast cell exhibited an unstained halo around the nucleus. The bone marrow contained numerous foci of mast cells with several chain formations (Fig. 4). Other organs from this animal were not available for microscopic study.

Only one of the 18 spontaneous ER growths that had been studied microscopically was free from mast cells.

At 11 months of age, mouse 2477 had a spherical gray-white splenic tumor, 1.5 cm. in greatest diameter. The spleen itself measured only 0.5 by 1.5 cm. The neoplastic cells were like those in the tumors already described. Figure 5 shows epithelium-like masses of these cells, in places with reticular arrangements. There were large numbers of hemopoietic cells in the spleen, predominantly erythroblasts, but no mast cells.

The ER cells of transplanted tumors were similar in appearance to those of spontaneous tumors (Figs. 6 to 9), and the relation of mast cells to tumor cells in transplanted neoplasms was the same as in the spontaneous neoplasms. The difference in the mast cell density between peripheral and central parts of the tumor was more marked in the transplanted than in the spontaneous tumors.

#### TRANSPLANTATION OF ER (EPITHELIUM-LIKE, RETICULUM-CELLED) SPLENIC TUMORS

Because of the unusual histologic character of the ER splenic tumor and its constant relation to mast cells, repeated efforts have been made during the past 8 years to secure a readily transplantable tumor of this type. This growth has thus far been encountered only in Ak mice and their hybrids, and in man.<sup>7</sup> Of six attempts at transplantation of spontaneous ER tumors, three were successful. The results of the transplantation experiments are summarized in Table II.

There were two interesting features in these transplantation studies: The unusually slow growth of this tumor, which has not changed in the course of several successive passages; and the adherence to the charac-

TABLE II  
*Attempted Implantations of Spontaneous ER Splenic Tumors*

Donor no.	No. of		Route of transplantation	Treatment of recipient	Result of transplantations†		Latent period
	Passages	Subpassages			Female	Male	
1147	II	5	Subcutaneous	Pre-irradiated	33/1	72/11	months 2-8
			Subcutaneous			12/2	
			Intrasplenic	Pre-irradiated	2/0	7/0	3-4
			Intrasplenic		3/0	3/0	
			Intra-ocular			5/0	
			Intravenous			8/0	
464	5	3	Subcutaneous	Pre-irradiated	16/9	3/2	4-6
			Subcutaneous		5/0	1/1	5
			Subcutaneous*			9/3	
300		1	Subcutaneous†		9/2		4.5-6
			Subcutaneous		4/0	8/0	
495	0	0	Subcutaneous*		3/0	12/0	
39	0	0	Subcutaneous†		3/0	3/0	
			Subcutaneous*		3/0	3/0	
2477	0	0	Intrasplenic	Castrated	2/0	2/0	
			Intrasplenic		1/0	1/0	
			Subcutaneous			2/0	
			Subcutaneous	Castrated	2/0		

\* and intraperitoneal.

† and intraperitoneal and intravenous.

‡ The figures in these two columns indicate the number of mice injected over the number of positives.

teristic cell types, namely, to epithelium-like reticulum cells with intervening mast cells.

The latent period, which is the interval between implantation of fragments of the tumor into the subcutaneous tissue and the appearance of a palpable nodule, varied between 2 and 8 months and averaged about 5 months. For example, in passage IIb of strain 1147, one mouse had a palpable tumor 52 days after injection, another only at 165 days. The latter tumor was excised at 281 days when it measured 2 by 2 by 1 cm. The transplanted tumors were invariably slow-growing and the animals had to be sacrificed when the tumor measured 1 to 2 cm. in greatest diameter because of the liability of the host animals to develop leukemia. Metastases have never been observed and removed tumors did not recur.

Normal males seemed slightly more susceptible (17 per cent) than normal females (10 per cent) to subcutaneous implantation of the tumor. Attempts at transmission by routes other than subcutaneous (intravenous, intrasplenic, and intra-ocular routes) made in a small number of mice were unsuccessful.

The difficulties of carrying on such strains by successive transplantations are indicated in Table II. Only the first strain listed is still alive, and five successive passages of this strain failed to increase the percentage of successful transplantations or to shorten the period of latency. Pre-irradiation did not enhance susceptibility to these neoplasms as it did to many others.

#### ABSENCE OF MAST CELLS IN FOUR SPLENIC TUMORS OTHER THAN THE DESCRIBED ER TYPE

Four splenic neoplasms other than the type described were studied for comparison. None of them contained mast cells. One was fibroma-like; the other three resembled monocytoma or histocytoma. One of the latter was in an animal which had neoplastic cells in the liver and another in a mouse with generalized leukemia.

#### MAST CELLS IN AND ABOUT MISCELLANEOUS NEOPLASMS

Neoplasms in general are free from mast cells. Luteoma is an exception (Table III). In some luteomas the number of mast cells exceeded that in the ER tumors (Fig. 10). None of the epithelial tumors examined contained them but they were encountered in several sarcomas and in an epithelioma.

The accumulation of mast cells around a tumor is more common. As Table III shows, they were frequent about some granulosa cell tumors and about some carcinomas and sarcomas. The transplanted granulosa cell tumors of strain I, about which no mast cells were seen, were of the folliculoid type. Tumors of strain IV had a diffuse pattern and those of strain V had areas of both diffuse and folliculoid types. The behavior of the mixed strain XI was noteworthy. This strain had lines with tumors of either or both granulosa and lutein cells. The mast cells invariably surrounded the granulosa cell masses but not the lutein cells.

Two of the carcinomas about which mast cells accumulated originated in the mammary gland. Those with no mast cells were 3 epidermoid carcinomas of the skin and 1 lacrymal gland carcinoma. The transplanted carcinomas with no mast cells were anaplastic, derived from endometrium. The 2 leukemias with a few mast cells about the leukemic infiltrations were monocytic; other monocytic, lymphoid, and myeloid leukemias did not contain mast cells. The endotheliomas were transplanted growths with rich capillary formations containing red blood corpuscles. One of the thymic sarcomas was spontaneous, the others were transplanted; the mast cells were found in the latter. The other

sarcomas were: 2 liposarcomas, 3 fibrosarcomas, 1 methylcholanthrene-induced and 1 benzpyrene-induced fibrosarcoma.

TABLE III  
*Mast Cells in and about Varied Neoplasms*

Neoplasm	No. examined	No. with mast cells		Mast cell density*	
		About neoplasm	In neoplasm	About neoplasm	In neoplasm
Transplanted ovarian tumors:					
Luteomas, strain IX	102	52	52	7-19	5-80
Granulosa cell tumors, strain I	9	0	0		
Granulosa cell tumors, strain IV	12	10	0	7-15	
Granulosa cell tumors, strain V	7	5	1	7-10	18
Mixed tumors, strain XI	9	5	0	10-14	
Choriocarcinoma-like line of strain IV	23	14	0	7-18	
X-ray-induced ovarian tumors:					
Tubular adenoma	14	6	5	5	7
Granulosa cell tumor	7	0	0		
Luteoma	7	0	2	3	
Carcinomas:					
Spontaneous	7	2	0	7-9	
Transplanted	9	0	0		
Adenomas, pulmonary, spontaneous	15	0	0		
Sarcomas:					
Miscellaneous, spontaneous	6	0	0		
Miscellaneous, induced	2	0	1		8
Thymic sarcomas	4	3	1	7-10	4
Endotheliomas, transplanted	3	3	1	10-50	12
Leukemias, miscellaneous	44	2	0	5-9	

\* The mast cell density is expressed by the number of mast cells in an 0.3 mm.<sup>2</sup> field of the section. The luteomas<sup>9</sup> and some transmitted granulosa cell tumors<sup>10</sup> have been described elsewhere. Description of the other granulosa cell tumors and of the choriocarcinoma-like growths will follow.<sup>11</sup>

The choriocarcinoma strain had, in the first few passages, cells of the two types indicative of this neoplasm. In the sub-passages these were replaced by endothelium-like cells which caused massive and occasionally exsanguinating hematomas. It seems probable that this is an unusual hemangio-endothelioma.<sup>11</sup>

All x-ray-induced ovarian tumors listed had extended into the periovarian adipose tissue. Of 13 additional tubular adenomas that were limited by the ovarian capsule, only one had mast cells present in the hilar region. In these tumors the mast cells were most numerous about ceroid cell masses and about tubules derived from the germinal epithelium. Of 5 additional granulosa cell tumors that were limited by the capsule, none had mast cells; of 4 additional luteomas, one had mast cells.

Table III warrants no generalization and gives no clue as to why mast cells accumulate in and about some tumors and not about others.

### *Characteristics of Mast Cell Infiltration in Neoplasms*

The distribution of mast cells in luteomas was, in general, similar to that in splenic ER tumors (Figs. 10 and 12), but in luteomas the accumulation of mast cells in the connective tissue was more conspicuous. The mast cells in luteomas were larger (about 18  $\mu$  in diameter) than in splenic tumors (about 11  $\mu$  in diameter) and, as in ER tumors, they sometimes were seen in capillaries (Fig. 12). As in ER splenic tumors,



the mast cells were in direct contact with the neoplastic cells. An occasional luteoma cell adjacent to mast cells stained metachromatically (Figs. 11 and 12), as did the granules of the mast cells; however, this material showing metachromasia was diffusely distributed in the cytoplasm of the luteoma cells.

Since most tumors studied were in the subcutaneous tissue, it is noteworthy that the number of mast cells in the subcutaneous tissue of the stock of mice studied ranged between two to five in an area  $0.3 \text{ mm.}^2$ , and that the mast cells were concentrated in the adventitia of vessels, in the peri-acinous areas of the mammary gland, and around hair follicles.

In transplanted tumors the mast cell increment was localized in areas where mast cells normally are found. In tumors extending into the subcutaneous fatty tissue, mast cells were especially abundant in connective tissue strands that separated two tumor nodules. The increment of mast cells occurred in foci and was irregularly spotty in distribution. The mast cells in tumors were slightly larger than in the normal skin. The density of granules and their affinity for dyes varied greatly in both. A substance staining metachromatically was not noted outside the mast cells.<sup>12</sup>

## DISCUSSION

### *Character of Mast Cells in the Tumors Studied*

Are mast cells neoplastic or hyperplastic? There is no doubt about the neoplastic character of epithelium-like reticulum cells since they multiply progressively by mitotic division with no apparent restraint. The mast cells infiltrating this growth do not appear to be neoplastic. Masses of ER cells can exist without mast cells but the presence of the latter is largely dependent on ER cells. Division forms of mast cells are not seen in small ER nodules. Mast cells are more numerous about strands of stromal connective tissue of the tumor than amidst tumor cells. Furthermore, mast cells are equally abundant in certain luteomas and occur occasionally in tumors of other types.

The known neoplasms of mast cells are monomorphous with admixture of a few other cells of the type encountered in most neoplasms.<sup>1,8,13,14</sup>

Immature mast cells have been well described by Oliver, Bloom, and Mangieri<sup>14</sup> in mastocytomas of dogs. These resemble adult mast cells but have fewer and smaller granules. The ER cells, unlike these mast cell precursors, are free from granules as viewed in the phase microscope and in Giemsa-stained preparations; transitional forms between ER cells and mast cells were never seen.

The data indicate that some tumors stimulate mast cell proliferation or attract mast cells. It is highly probable that this is brought about by some substance present in the tumor or by a metabolite of the tumor cell. There is no known common denominator of neoplasms which contain mast cells. The regular association of ER tumors and luteomas with mast cells suggests experimental studies on the induction of mast cell accumulations by means of subcutaneous injections of cell-free extracts of these tumors.

One exceptional case is noteworthy. In mouse Akh 152, the spleen and bone marrow contained numerous dense foci of mast cells which were also concentrated in the periphery of ER tumors. These mast cell foci resembled those interpreted by Deringer and Dunn<sup>8</sup> as neoplastic. If this interpretation is correct, this animal had two neoplasms which could have arisen independently, or the mast cell hyperplasia in ER tumors changed into neoplasia.

#### *Character and Transplantation Pattern of ER Cells*

These epithelium-like splenic tumors originally were described as probably reticular.<sup>7</sup> However, this is uncertain. In the first place, they are unlike the usual reticulum cell sarcomas described in man and animals. These neoplastic cells resemble epithelial cells, notably liver cells, and there is a remote possibility that they are heterotopic epithelial cells.

In a study by LeCompte<sup>15</sup> of tumors of the carotid body, Figure 10 illustrates a tumor with an adenoma-like pattern in which the chief cells have a remarkable epithelium-like arrangement. Some illustrations in that article (Figs. 4, 5, 8, and 10) present a picture indistinguishable from that of our splenic tumors (Figs. 1, 5, and 6). It is conceivable that an organ, such as the spleen, concerned with regulation of blood volume, contains cells which function as do those of the carotid body, but to our knowledge such cells have not been described in the spleen. Further studies are desirable, including the use of tissue cultures and histochemical procedures, to establish beyond doubt the character of the ER cells.

The ER tumors are known only in Ak mice and their hybrids. The rarity of their occurrence (a few in a thousand) is noteworthy because this strain is highly inbred. Accordingly, a high percentage of ER tumors would be expected, or none at all. The difficulties of transplantation in the homologous highly inbred strains are likewise noteworthy.

*Histogenesis of Mast Cells*

The shape of mast cells and tumor cells, and the appearance of their nuclei and cytoplasm are entirely different and there are no transitional forms between the two. Therefore, the possibility of origin of mast cells from tumor cells can be excluded. Attracted by substances of the tumor cells, the mast cells either migrate into the tumor, or they proliferate in the tumor.

The highly controversial subject of the origin of mast cells has been ably reviewed by Michels.<sup>1</sup> Some workers derive mast cells from "reticulum" cells; others, from histiocytes, "resting" wandering cells, endothelium, "hemohistoblasts," fibroblasts, lymphocytes, and plasma cells.<sup>1</sup> The possibility of origin of mast cells in the bone marrow can be excluded since this organ is almost free from mast cells in mice bearing ER tumors. Our material likewise failed to disclose any evidence of migration of mast cells from blood vessels into the tumor or surrounding connective tissue.

The arguments and observations cited by Michels<sup>1</sup> apply also to the origin of mast cells in luteomas and other tumors. Division figures are scanty and the relation to connective tissue is evident in all. In general, it seems that mast cells increase in connective tissue about the tumor, and later become incorporated in it and continue to proliferate or accumulate in the connective tissue stroma in and about the tumor.

In both ER splenic tumors and luteomas there is a close relationship between tumor cells and mast cells in that the mast cells surround the tumor cells by cytoplasmic extensions. In luteomas a metachromatic staining is noted in a few cells adjacent to mast cells. This is not due to imbibition of dissolved granules or to insufficient dehydration of the sections (false metachromatic staining of Sylvén<sup>12</sup>) because the color is within the boundaries of the luteoma cells.

There is much discussion in the older literature concerning the motility of mast cells,<sup>1</sup> but Paff, Bloom, and Reilly<sup>16</sup> recently have demonstrated a slow ameboid motion of mast cells by means of motion pictures. Earlier, Lehner<sup>17</sup> had observed in the mesentery and other tissues of the frog, retraction of cytoplasmic processes and movement of granules in mast cells.

The relationship between mast cells of tissue and blood has not been established definitely, although most observers believe that they are unrelated.<sup>1,18</sup> In a transplantable mast cell leukemia of mice<sup>19</sup> the bone marrow was a frequent site of mast cell proliferation and, unlike cells of mastocytomas, the neoplastic mast-myelocytes behaved as other leu-

kemic leukocytes. The bone marrow is almost entirely devoid of tissue mast cells in both normal mice and in mice bearing ER tumors rich in mast cells. Both shape and size are variable, as exemplified by the behavior of monocytes which are spherical in the blood, assuming microglia-like forms in tissues and large epithelioid forms in reactive processes. Accordingly, shape and size are not trustworthy characteristics to distinguish mast cells of tissue from those of blood. Although mast cells of tissue and blood appear different morphologically and histogenetically, their metachromatic cytoplasmic granules appear similar and functionally these two types may be related. The term "heparinocyte" has been suggested<sup>8</sup> for mast cells of the blood, but this term should include mast cells of tissue as well; or, according to Jorpes,<sup>3</sup> should preferably be given only to mast cells of tissue.

Very recently, Barnard<sup>20</sup> mentioned coincident hyperheparinemia, basophilic myelocytosis, and estrapenia in 3 cases of purpura. He postulated that hemal mast cells of marrow origin are responsible for heparin production and that the heparin level regulates thromboplastin production by the bone marrow. Such an equilibrating mechanism would satisfactorily explain failure of hemorrhagic tendencies in animals bearing tumors rich in mast cells, although it is equally conceivable that the mast cell proliferation is secondary to a hypothetic thromboplastic substance elaborated by tumors.

### *The Function of Mast Cells*

Opinions on the supposed function of mast cells have been well reviewed by Michels<sup>1</sup> and only a few will be mentioned here. The recent discovery of the chemical composition of mast cell granules pushed aside the earlier, more or less speculative assumptions on the function of mast cells and linked them with two seemingly unrelated processes: (a) production of the ground substance of connective tissue, and (b) the blood clotting mechanism. There is suggestive evidence for both. These two functions appear unrelated unless it is postulated that the polyester sulfuric acid contributed by mast cells to the connective tissue helps to maintain the semi-fluidity or gel-like character of the connective tissue, thus facilitating migration of leukocytes and vital processes.

Staemmler<sup>21</sup> is the strongest proponent of the view that mast cells are related to the intercellular connective tissue substance. He noted a direct correlation between the amount of loose connective tissue and the mast cell content of tumors, in line with his thesis that mast cells aid the formation of connective tissue by contributing "mucin" to it. The trans-

plantable ER tumors in which we noted an abundance of mast cells may be related to reticulum cells, but other more typical reticulum cell tumors did not contain mast cells. The transplantable luteomas characteristically rich in mast cells were relatively poor in connective tissue.

In the opinion of Sylvén,<sup>12</sup> mast cells give to the collagenous fibers a "free chromotrope substance," a polyester of sulfuric acid, during their breakdown, brought about by a proteolytic enzyme derived from the tumor. Sylvén found a mast cell increment and free chromotrope substance only in very rapidly growing parts of mesenchymal tumors. The mast cell increment in our ovarian tumors was not related to the state of differentiation or rapidity of tumor growth, and, as already stated, the luteomas rich in mast cells were unusually poor in connective tissue stroma.

It is noteworthy that a hemorrhagic tendency was not apparent in mastocytoma-bearing animals or in mice with tumors rich in mast cells. Heparin has been shown to inhibit cell growth (Zakrzewski<sup>22</sup> and Fischer<sup>23</sup>), and the opinion that mast cells inhibit tumor growth has been expressed by numerous investigators. Fromme<sup>24</sup> and others believed that mast cells aid the formation of connective tissue barriers about tumors and neutralize "toxins" of cancer cells.

The relation between carcinogens and mast cell increment has been the subject of numerous studies. Fabris<sup>25</sup> exposed mice to an atmosphere of finely pulverized tar. These animals developed nodules and circumscribed agglomerations of mast cells with plasma cells in the papillary layer of the skin, notably in the cheeks, shoulders, feet, ears, and inguinal region. Franco and Affonso<sup>26</sup> observed an immense increment of mast cells in the skin after 67 applications of tar. Schreus<sup>27</sup> painted mice with neutral tar oil and noted a nodule in subcutaneous tissue composed entirely of mast cells. Twort and Twort,<sup>28</sup> using oil and tar, obtained mast cell tumors in the skin near the dermis, one of which was invasive. Cramer and Simpson<sup>2</sup> observed a particularly marked mast cell increment about the benign epithelial hyperplasia occurring following methylcholanthrene treatment.

Thus it is observed that certain agents, including some carcinogens and some tumor cells, stimulate mast cells; the ability of certain tumor cells to do so seems to be a characteristic property of the tumor cells, but the agent directly responsible for mast cell stimulation is unknown as is the meaning of mast cells in tumors.

That the function of mast cells is more complex than mere elaboration of heparin is suggested by the recent findings of acid<sup>29</sup> and alkaline

phosphatases and cytochrome oxidase<sup>30</sup> in mast cell granules, and also sudanophile lipoids.<sup>29,31</sup>

If mast cells produce heparin, the question remains to be answered why some tumors contain them in abundance and others contain none. It is conceivable that metabolites or autolytic products of tumor cells promote blood clotting to a varying extent and that mast cells counteract these blood clotting substances.

Further chemical and hematologic studies are desirable to learn about the functions of mast cells. The transplantable tumors rich in mast cells may serve that purpose.

#### SUMMARY

A benign transplantable splenic tumor occurs in the Ak strain of mice, with neoplastic cells resembling epithelium. These cells are probably related to reticulum cells.

These neoplasms, both spontaneous and transplanted, are characteristically rich in mast cells. Histogenetically, the mast cells are not related to the neoplastic cells. They appear to be tissue cells not derived from the bone marrow or other hemopoietic organs.

Mast cells are characteristically present in certain neoplasms and are absent in others. Among the former are luteomas, both spontaneous and transplanted; among the latter are granulosa cell tumors, pulmonary adenomas, breast cancers, and malignant lymphomas.

The theory is favored that a dynamic equilibrium exists between blood clotting (thromboplastic) substances and the fluidity-maintaining substances (heparin). This gives a meaning to mast cell proliferation and explains why animals bearing tumors rich in mast cells do not exhibit hemorrhagic tendencies.

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[ Illustrations follow ]



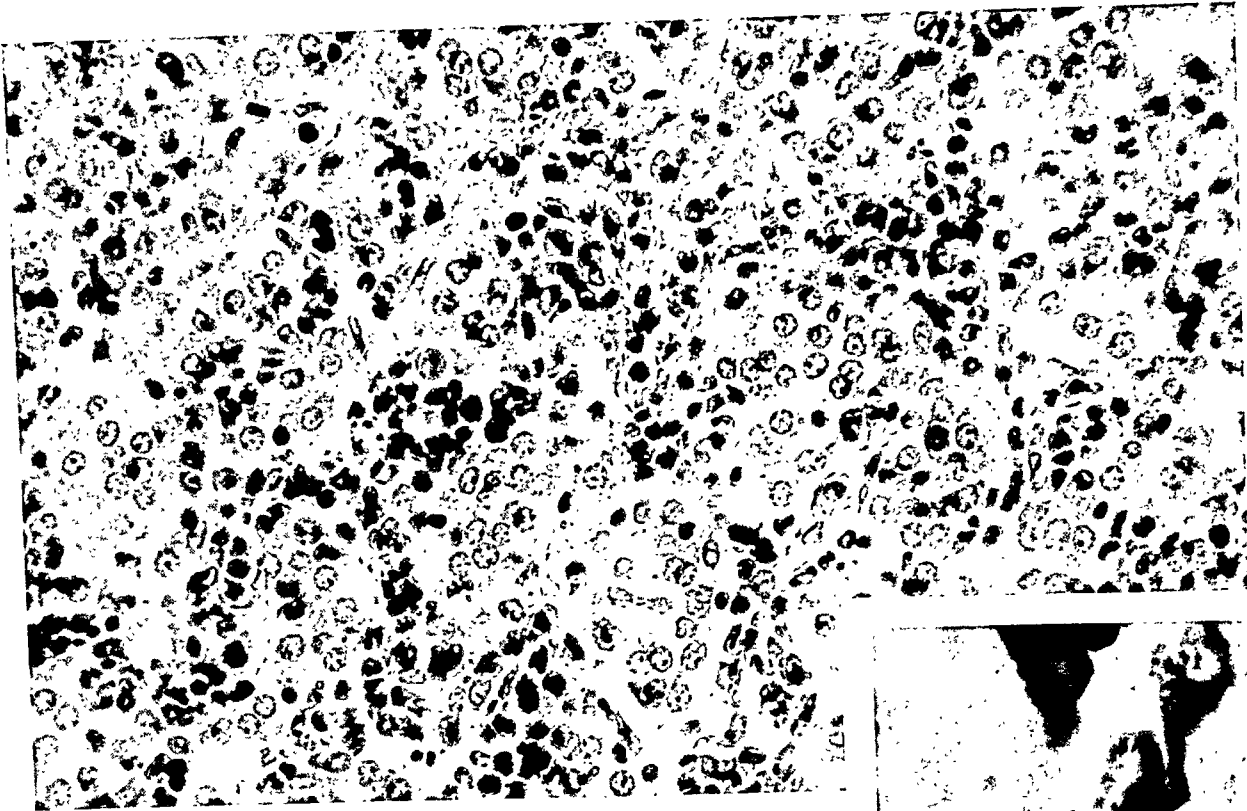
## DESCRIPTION OF PLATES

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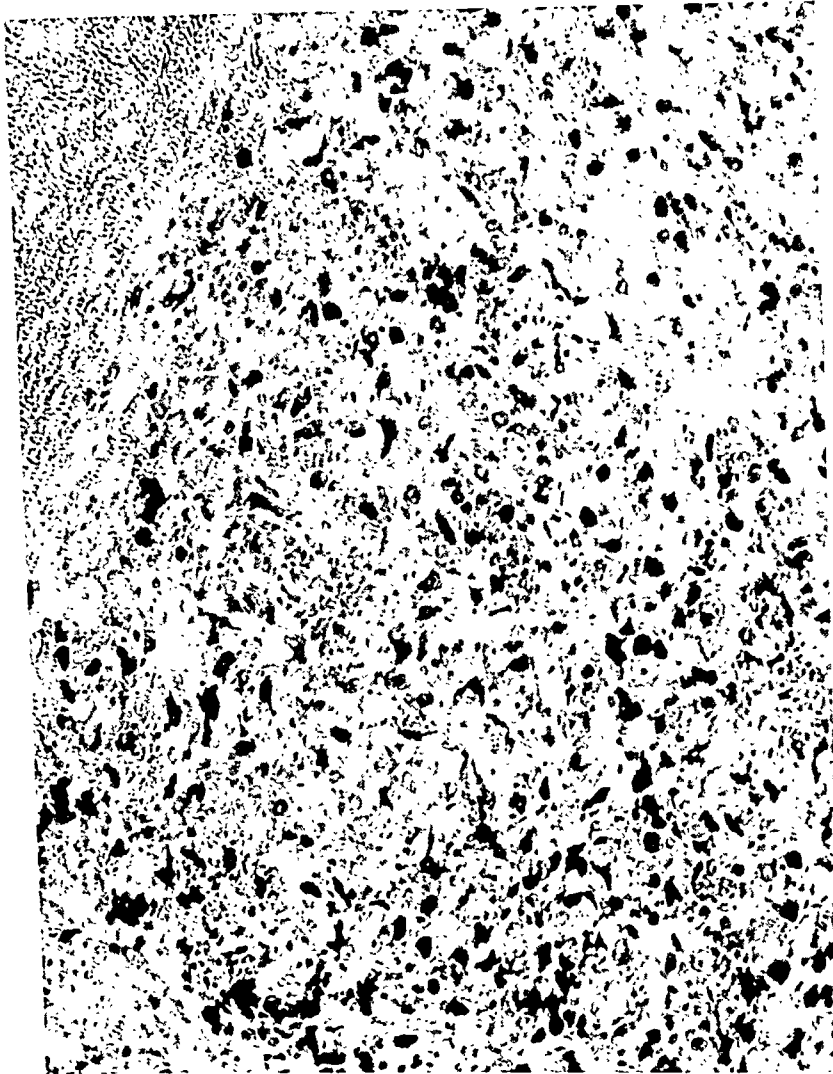
### PLATE 93

- FIG. 1. Spontaneous ER tumor in the spleen. Among the nests of tumor cells, erythropoietic and granulopoietic foci are seen. The mast cells are unstained. Hematoxylin and eosin stain.  $\times 450$ .
- FIG. 2. Mast cells in a spontaneous splenic ER tumor, showing variability in their shape and size. Giemsa's stain.  $\times 100$ .
- FIG. 3. An area from Figure 2, showing mast cells and a binucleated cell in the center of the field. Giemsa's stain.  $\times 950$ .
- FIG. 4. A chain of mast cells in the bone marrow of the only case (Akh 152) in which mast cells were abundant in this organ. Giemsa's stain.  $\times 950$ .

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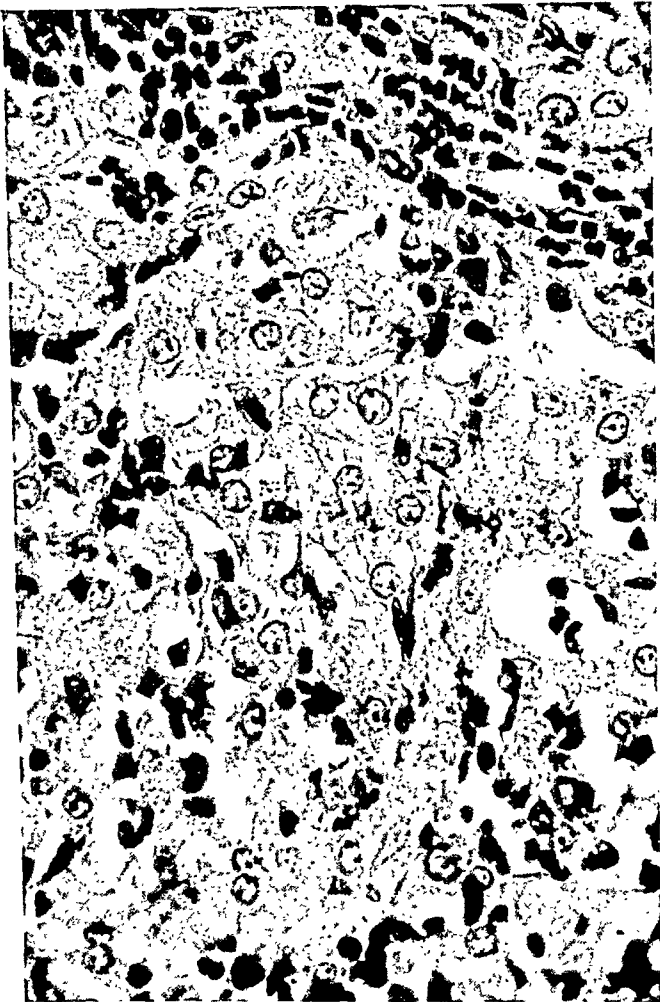
Bali and Furth

Splenic Tumor Rich in Mast Cells

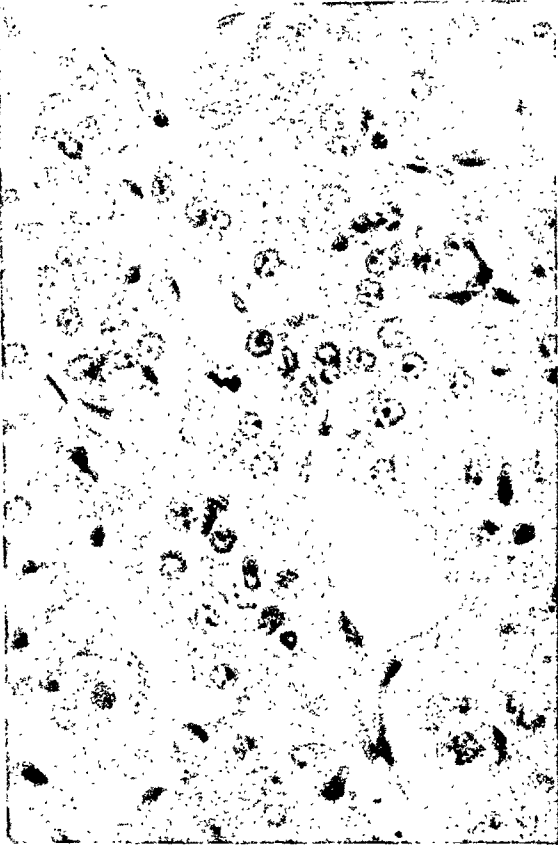
PLATE 94

- FIG. 5. Spontaneous splenic ER tumor, the only one that was devoid of mast cells. Hematoxylin and eosin stain.  $\times 570$ .
- FIG. 6. A subcutaneously transplanted ER tumor with neoplastic cells in trabecular arrangement, with wide capillary spaces among them. This picture resembles closely that of the carotid body tumor of Le Compte.<sup>15</sup> Hematoxylin and eosin stain.  $\times 850$ .
- FIG. 7. General appearance of a transplanted ER tumor. Hematoxylin and eosin stain.  $\times 100$ .
- FIG. 8. Two mast cells from a spontaneous splenic ER tumor that appear to be connected by cytoplasmic processes, a picture frequently encountered. Giemsa's stain.  $\times 950$ .
- FIG. 9. A subcutaneously transplanted ER tumor with a mitotic figure in the center. The epithelium-like appearance of the tumor cells is particularly well shown. Hematoxylin and eosin stain.  $\times 950$ .

5



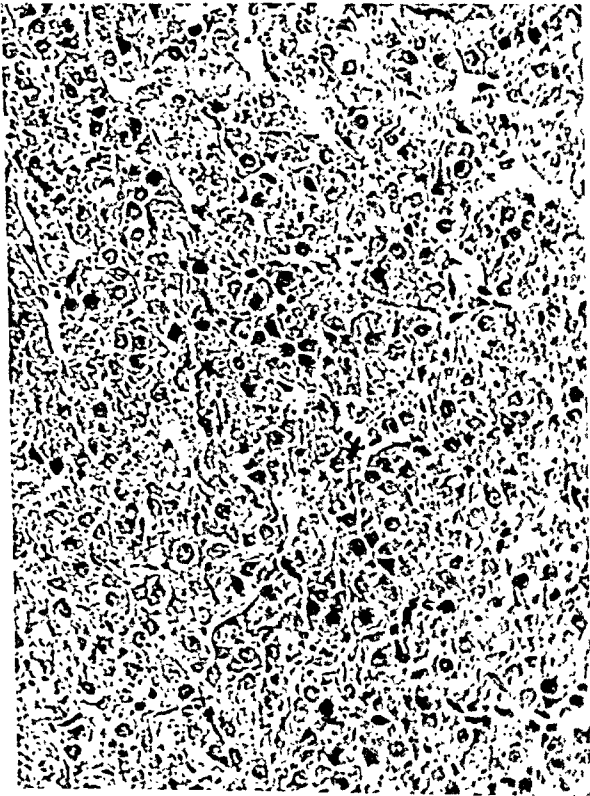
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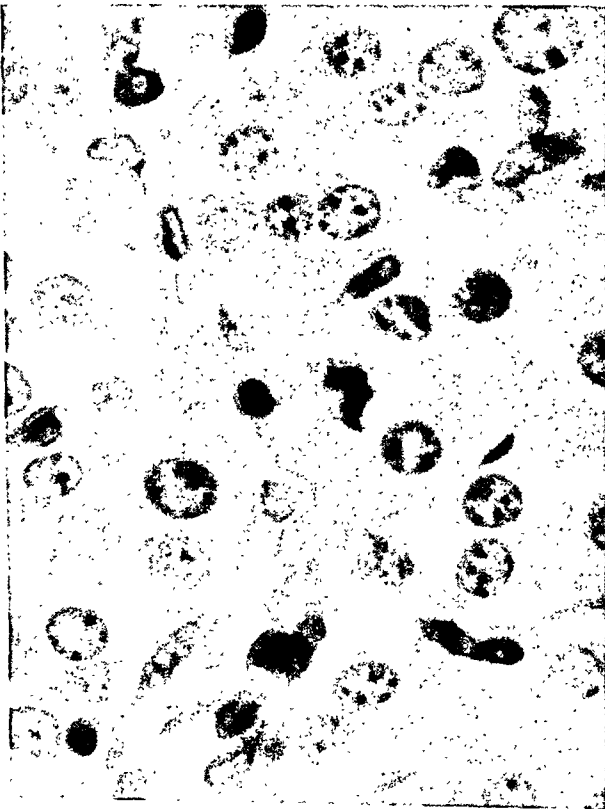
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Bali and Furth

Splenic Tumor Rich in Mast Cells

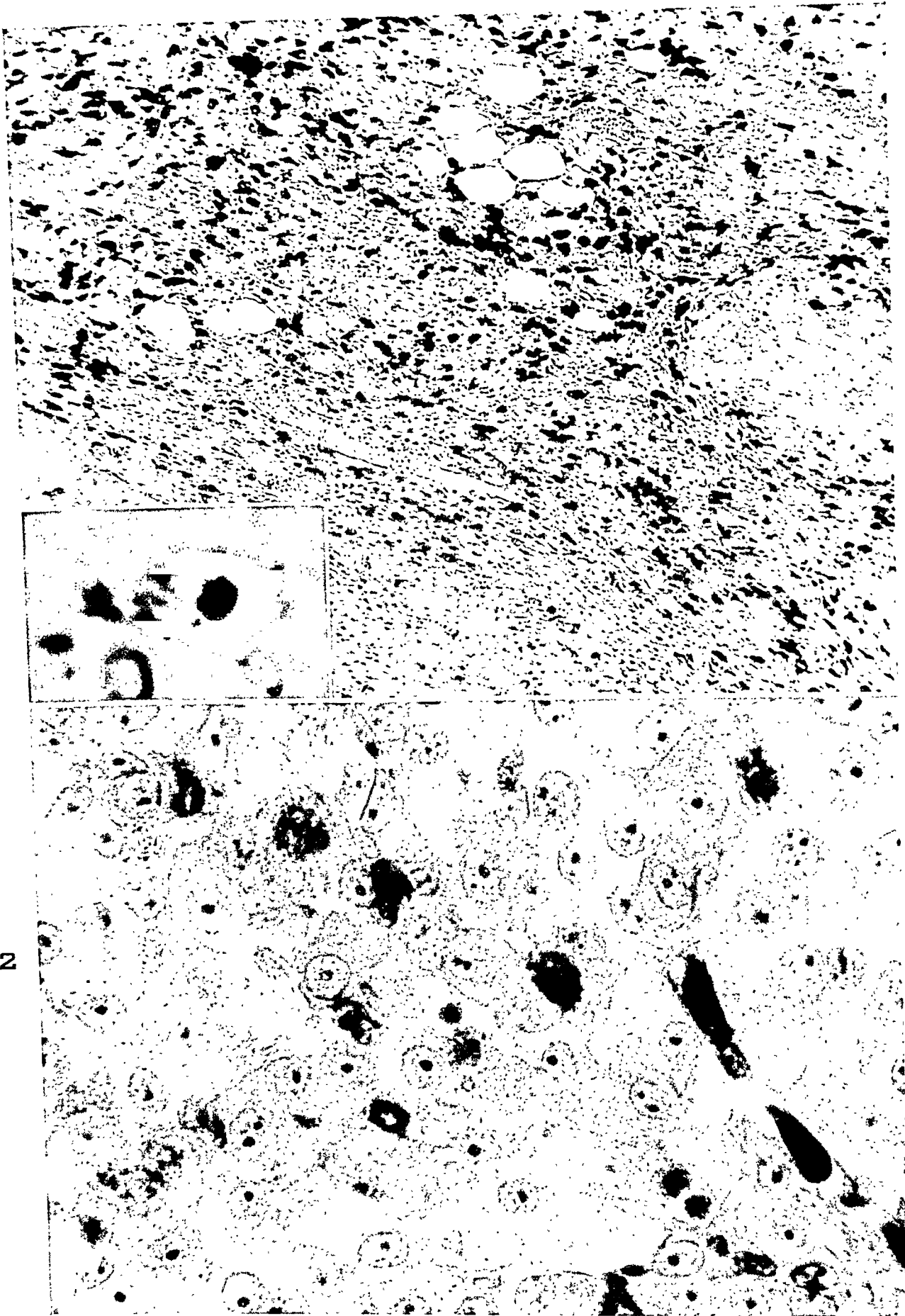
PLATE 95

- FIG. 10. Transplanted luteoma (strain IX) in subcutaneous fatty tissue. Mast cells are absent in the central parts of an apparently young tumor nodule close to the right margin of the field, but are abundant in the peripheral parts of the nodule and in other, presumably older, parts of the tumor. Giemsa's stain.  $\times 200$ .
- FIG. 11. A sharply demarcated, unstained vacuole in a mast cell in a luteoma. Giemsa's stain.  $\times 970$ .
- FIG. 12. Luteoma cells, metachromatically stained, adjacent to mast cells. The two mast cells in the right lower field are in a capillary. Giemsa's stain.  $\times 950$ .

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11

12





## SEBACEOUS GLANDS IN NORMAL AND NEOPLASTIC PAROTID GLANDS

### POSSIBLE SIGNIFICANCE OF SEBACEOUS GLANDS IN RESPECT TO THE ORIGIN OF TUMORS OF THE SALIVARY GLANDS\*

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The presence of structures resembling sebaceous glands in two parotid glands that contained adenomas has been mentioned in another publication.<sup>1</sup> In those cases it was observed that such structures arose from the intralobular ducts. In one instance, sebaceous glands were seen in an area of tumor formation as well as in the normal parotid tissue. However, in the other they were present only in the normal portion. The glandular structures were in all respects similar to the sebaceous glands found in areas where they are not associated with hairs. Their occurrence in parotid glands is not mentioned in any of the standard textbooks of histology. Search of the literature revealed two reports in which similar findings have been recorded. Hamperl<sup>2</sup> observed holocrine fat secretion (sebaceous-like transformation) in the excretory system in four submaxillary glands of 4 of 85 persons from whom he studied the uvular, sublingual, and submaxillary glands, and in one parotid gland. Hamperl stated that he found sebaceous-gland-like transformation in small intralobular ducts ending blindly. The ages of the persons in whom this transformation was found in the submaxillary glands were 6½, 9, 20, and 36 years, respectively, and the person whose parotid gland showed the sebaceous-gland-like transformation was 6 years old.

The only other report of sebaceous glands in the parotid gland is that of Hartz.<sup>3</sup> The individual in whom they were found was a 34-year-old woman who had a recurrent mixed tumor of the left parotid gland. The tumor was removed with the surrounding glandular tissue. The microscopic study of the specimen showed a typical "mixed tumor" and some normal parotid gland in which Hartz found small sebaceous glands originating from the striated or intercalated ducts. He had the impression that the sebaceous glands developed from short branches that ended blindly. This author pointed out that it was not known whether such structures "were present before the tumor and the first operation or whether those abnormal circumstances were the stimulus for the peculiar differentiation of the ductal epithelium."

Because of these findings it was thought that further study was neces-

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sary to determine (1) whether sebaceous glands are present in normal parotid glands, (2) whether they are present in parotid glands which are the site of a neoplastic process, and (3) whether or not the occurrence of such structures in the salivary glands could have any bearing on the explanation of the tumors occurring in these salivary glands. With these questions in mind, it was decided to study normal parotid glands, and those in which tumors had occurred.

### MATERIAL AND METHODS

The material for this study was divided into two groups: (1) tumors of the parotid gland around which there was normal salivary gland tissue, and (2) normal parotid glands obtained from autopsy material.

The first group consisted of prepared sections from 249 tumors of the parotid gland of which only 133 showed some normal salivary gland tissue about the tumor. These latter slides were used for the present study. With the exception of one case from Memorial Hospital, New York City, all came from the Department of Pathology of the University of Michigan. They represented the tumors of the parotid gland diagnosed during the preceding 15 years, which will be considered together and will be referred to as group 1 throughout this communication (Table I).

TABLE I  
*The Material of Group 1 (Tumor Cases) with Percentage of Those That Had Sebaceous Glands*

Number of tumors	249
Number of tumors with normal parotid gland attached	133
Number of cases with sebaceous glands	33
Percentage of cases with sebaceous glands of those with parotid gland attached	24.8

The amount of normal parotid tissue present varied considerably, but as a rule it was not very abundant. In order to be included in this study, it was required that at least one of the sections show 25 mm.<sup>2</sup> of normal salivary gland. All of the sections from this group were stained with hematoxylin and eosin.

The second group was composed of 100 normal parotid glands obtained from 51 persons who died from various causes, including accidents and violence. These were unselected individuals on whom autopsies were performed at the University Hospital. Their ages ranged from 7 months to 76 years. Thirty-three were males. Both parotid glands were available for study from 49 individuals and from each of 2, only one gland could be obtained. The amount of parotid tissue removed from each person was about two-thirds of each gland in most cases. Formalin was used to

fix 80 of the 100 parotid glands and the remaining 20 were fixed in absolute alcohol. An average of two blocks of tissue were imbedded in paraffin from each gland and five sections obtained from each block were stained with hematoxylin and eosin. Hortega's silver carbonate nuclear stain on frozen sections<sup>4</sup> was done in every case in which sebaceous glands were found, provided the material had been fixed in formalin. This was followed by scharlach R staining for fat in every section which showed sebaceous glands in the silver impregnation. Mayer's mucicarmine stain was used on material fixed either in formalin or in alcohol, and Best's carmine stain when the sections showed sebaceous glands and the material had been fixed in absolute alcohol.

### MICROSCOPIC FINDINGS

The microscopic findings were similar in both groups. The parotid glands presented the normal tubulo-acinar structure and they varied only in degree of atrophy, as recorded in Table II. Most of the ducts were normal, but in areas changes had taken place in some branches of the striated ducts or more frequently in the intercalated ducts. These branches or ducts usually were short and had blind ends, instead of branching normally into intercalated ducts or ending in glandular acini. The blind ducts were lined by low-cuboidal epithelium that progressively changed into the stratified squamous type. This change was sometimes very abrupt.

Some of these ducts were curved, forming small hooks which often ended in saccular or alveolar epithelial structures which varied in size from 50 to 350  $\mu$ . They were spherical, ovoid, or elongated, and were composed of a well defined basal membrane over which lay a row of cuboidal or flattened epithelial cells (Figs. 1 to 8). Sometimes these cells formed small groups composed of several layers situated about the periphery of the alveolus or toward the lumen of the duct (Figs. 2, 3, 4, and 8). Their nuclei were round or oval and larger than those of the cells of the parotid acini or of secretory or intercalated ducts (Figs. 1, 2, 5, and 6). They usually showed a small nucleolus, although occasionally two nucleoli could be seen. The cells increased in size toward the center of the alveolar mass, where they were spherical, polyhedral, or irregular in shape. Their cytoplasm was vacuolated, the vacuoles being larger in the cells more centrally situated or close to the opening of the alveolus into the lumen of the duct (Figs. 1 to 8). Some of the nuclei of these cells were similar to those of the peripheral cells, but others were irregular and pyknotic. In some cells no nuclei could be seen. In many cases the

TABLE II  
Data on the Cases in Which the Parotid Glands Were the Site of Tumors and in Which  
Sebaceous Glands Also Were Found

No.	Sex	Age	Side	Diagnosis of the tumor	Amount of parotid gland available	State of the parotid	Sebaceous glands
1	F	23	?	Mixed tumor	Small piece	Normal	+
2	F	33	?	Mixed tumor	Small piece	Slight atrophy	+
3	F	?	?	Muco-epidermoid tumor	Very small piece	Slight atrophy	+
4	F	60	?	Adenoma	Very small piece	Moderate atrophy	+
5	F	68	?	Mixed tumor	Very small piece	Moderate atrophy	+
6	F	52	Left	Muco-epidermoid tumor	Small piece	Normal	+
7	M	25	?	Mixed tumor	Small piece	Normal	+
8	M	31	Left	Mixed tumor	A piece	Slight atrophy	+
9	M	42	Left	Mixed tumor	Small piece	Moderate atrophy	+
10	M	21	Right	Mixed tumor	Small piece	Slight atrophy	+
11	M	24	Right	Mixed tumor	Very small piece	Slight atrophy	+
12*	M	50	Left	Adenoma	Small piece	Slight atrophy	+
13	F	19	Left	Mixed tumor	Small piece	Slight atrophy	+
14	F	31	Left	Mixed tumor	A piece	Slight atrophy	+
15	F	25	Right	Carcinoma	A piece	Moderate atrophy	+
16	M	5	Left	Carcinoma	Small piece	Slight atrophy	+
17	M	34	Left	Muco-epidermoid tumor	Small piece	Slight atrophy	+
18	M	21	Left	Muco-epidermoid tumor	A piece	Slight atrophy	+
19	F	38	Left	Muco-epidermoid tumor	Small piece	Slight atrophy	+
20	M	36	Left	Carcinoma	Small piece	Moderate atrophy	+
21	M	34	Right	Muco-epidermoid tumor	Small piece	Slight atrophy	+
22	M	57	Right	Mixed tumor	A piece	Moderate atrophy	+
23	M	44	Right	Muco-epidermoid tumor	A piece	Moderate atrophy	+
24	F	17	Left	Carcinoma	A piece	Moderate atrophy	+
25	F	34	Left	Carcinoma	A piece	Slight atrophy	+
26	M	28	Right	Mixed tumor	Small piece	Normal	+
27	M	77	Left	Mixed tumor	Small piece	Atrophy	+
28	M	46	Right	Mixed tumor	Small piece	Moderate atrophy	+
29	F	40	Right	Mixed tumor	Small piece	Moderate atrophy	+
30	F	47	Right	Mixed tumor	A piece	Slight atrophy	+
31	F	37	Left	Muco-epidermoid tumor	Very small piece	Atrophy	+
32	M	57	Right	Carcinoma	Very small piece	Slight atrophy	+
33	F	51	Left	Mixed tumor	Small piece	Moderate atrophy	+
					A piece	Slight atrophy	+

\* Case from Memorial Hospital, New York City.

large cells with shrunken nuclei were separating into the lumen of the duct, forming a holocrine secretion (Figs. 1, 4, and 7). Mitotic figures were seen only in the cells of the periphery of the alveoli (Fig. 7). The alveoli usually were single at the end of the blind ducts but sometimes two or three alveoli opened into a duct to form a simple branched gland (Fig. 8). In frozen sections, stained with Hortega's silver carbonate, it was seen that the empty vacuoles, observed in the paraffin sections, were filled with a translucent, refringent substance. When these same sections were stained subsequently with scharlach R, the substance contained in the vacuoles varied from orange to red. Sections from paraffin-imbedded tissue stained with Mayer's mucicarmine and Best's carmine failed to show mucin or glycogen in the vacuoles. The presence of sebaceous-gland-like structures was demonstrated in frozen sections from only 14 of 22 parotid glands which showed them in the paraffin sections and which were saved in formalin, although hundreds of sections were studied from the remaining 8 cases.

#### COMPARATIVE STUDY OF THE FINDINGS IN BOTH GROUPS

From the microscopic findings, it was evident that the epithelial structures found in the parotid glands had morphologic and tinctorial characteristics similar to those of sebaceous glands, particularly of the kind seen in areas where such glands are not associated with hair. These structures will be designated as sebaceous glands in the discussion which follows.

The findings and certain clinical data for the patients in whom the sebaceous glands were found are summarized in Tables II and III, but some of the facts recorded there should be emphasized.

*Incidence of Sebaceous Glands.* Thirty-three parotid glands of group 1 showed sebaceous glands and 28 in group 2, which represents 24.8 per cent and 28 per cent, respectively. In group 2, sebaceous glands were found in both parotid glands in 11 persons and in one parotid gland only in 6 persons, making a total of 17. In other words, 33 per cent of the persons whose parotid glands were examined at autopsy showed sebaceous glands.

*Incidence in Relation to Sex.* The 33 parotid glands of group 1 that showed sebaceous glands were from 17 males and 15 females, with one person whose sex was not recorded. In group 2, 12 of those in whom sebaceous glands were found were male and 5 female. This apparent preponderance in the male sex is not significant, because 33 persons of this group were male and only 18 female.

*Incidence in Relation to Age.* The occurrence of sebaceous glands

TABLE III  
Data on the Autopsy Cases in Which the Parotid Glands Showed Sebaceous Glands

No.	Sex	Age	Cause of death	State of the parotid	Sebaceous gland	Remarks
1*	M	70	Bronchial asthma, retroperitoneal hemorrhage	Moderate atrophy	+	Positive fat stain
2	M	67	Myocardial infarction, squamous cell carcinoma of tongue and floor of mouth	Moderate atrophy	++	Positive fat stain
3	M	54	Hypernephroma of left kidney with multiple metastases, diabetes	Slight atrophy	+	No sebaceous gland found in frozen sections
4*	M	76	Carcinoma of stomach, bronchopneumonia	Marked atrophy	+	No sebaceous gland found in frozen sections
5*	F	21	Spongiblastoma polare, acute purulent lobular pneumonia	Slight atrophy	+	Positive fat stain
6*	M	22	Subdural hematoma, multiple fractures	Moderate atrophy	+	No sebaceous gland found in frozen sections
7*	F	49	Carcinoma of the breast, with multiple metastases	Slight atrophy	++	No sebaceous gland found in frozen sections
8	F	64	Third-degree burns, carcinoma of the thyroid	Slight atrophy	+	No sebaceous gland found in frozen sections
9	M	43	Myelosarcomatosis, bronchopneumonia	Slight atrophy	++	Positive fat stain
10	M	25	Chronic glomerulonephritis, uremia, peptic ulcer	Normal	+	Sebaceous glands negative for glycogen and mucin
11	F	15	Polycythemia, massive infarction of occipital and parietal portion of brain	Slight atrophy	+	Sebaceous glands negative for glycogen and mucin
12	M	13	Medulloblastoma of cerebellum invading meninges and spinal cord	Slight atrophy	+	Positive fat stain
13	F	36	Dicoumarol poisoning	Slight atrophy	+	Positive fat stain
14	M	6	Encephalitis	Normal	++	Positive fat stain
15	M	21	Epidural hemorrhage	Normal	++	Positive fat stain
16	M	19	Bullet wound	Normal	++	Positive fat stain
17*	M	50	Multiple bullet wounds	Normal	+	Positive fat stain

\* Cases in which only one parotid gland showed sebaceous glands.

had no relation to age, as can be seen clearly in Table IV, which shows that the youngest person was 5 years old and the oldest 77 years in group 1, and 6 years and 76 years in group 2. The concentration noted from the second to the sixth decade in group 1 probably cannot be considered significant since that is a period in which most salivary gland tumors occur.

TABLE IV  
*Age Distribution by Decades of Cases with Sebaceous Glands*

Age	Tumor cases* (group 1)	Autopsy cases (group 2)
1-10	1 (Youngest 5 years)	1 (Youngest 6 years)
11-20	2	3
21-30	7	4
31-40	10	1
41-50	5	3
51-60	5	1
61-70	1	3
71-80	1 (Oldest 77)	1 (Oldest 76)

\* Age not known for 1 case.

*Sebaceous Glands and Glandular Atrophy.* There was no association between atrophy of the parotid gland and the occurrence of sebaceous glands since they were found in parotid glands showing all degrees of atrophy or no atrophy at all.

*Number and Degree of Development of the Sebaceous Glands.* The study of the material in relation to frequency and to degree of development showed that sebaceous glands were more numerous in the cases of group 1, in which they also reached a larger size and sometimes presented a branched appearance, while in group 2 they were less numerous, smaller, and only one branched gland was seen (Figs. 14 to 20). The average number of sebaceous glands found in the sections from each case of group 1 was 4.5, while in group 2 it was 2.5. These figures, although significant, do not adequately express the preponderance clearly observed in the study of the material, for it was noticed that there was greater frequency of occurrence of two or more sebaceous glands in one microscopic field in cases from group 1 (Figs. 9 to 13), while in group 2 on only two occasions were two sebaceous glands seen in one field (Fig. 14). This lack of agreement between observation and tabulation can be explained by the fact that the amount of normal parotid gland available for study from each case of group 1 was often one or two small sections, while in cases from group 2 at least 10 sections obtained from two different blocks were studied. In regard to size, the largest sebaceous glands were seen in parotid glands of group 1 in which they reached 350  $\mu$  in diameter.

*Relation of Sebaceous Glands and Type of Tumor.* Sebaceous glands were found in association with 17 mixed tumors, 8 neoplasms of the muco-epidermoid type recently separated from the so-called mixed tumor group,<sup>5</sup> 6 carcinomas, and 2 adenomas. The great frequency with which sebaceous glands were present in parotid glands that were the site of muco-epidermoid tumors should be noticed. This can be brought out better by two facts. First, examples of this type comprised 24 per cent of the tumors associated with sebaceous glands. This figure is very high if one considers that the incidence of the muco-epidermoid tumor in relation to all salivary gland tumors is 5+ per cent.<sup>5</sup> Second, sebaceous glands were found in 8 of 11 cases of muco-epidermoid tumor in which there was normal parotid gland present; or, in other words, they were found in 73 per cent of the parotid glands that were the site of such tumors. The association of sebaceous glands and carcinoma in the same parotid gland also seems to be of interest, because this occurred in 18 per cent of the cases of group 1 in which sebaceous glands were seen. This is a higher incidence than that of carcinoma in relation to mixed tumors, which was estimated to be 14+ per cent in the material studied for this report. This figure comes close to the percentage for malignant tumors of the parotid gland found by Stein and Geschickter,<sup>6</sup> which was 17 per cent, and the 15+ per cent obtained from the cases studied by Benedict and Meigs,<sup>7</sup> who had 30 examples of carcinoma in 203 cases of parotid tumors.

#### COMMENTS

If it be accepted that the epithelial structures found in parotid glands are truly sebaceous glands, it becomes necessary to explain how these accessory skin structures happen to be present in this location. Their occurrence in the mucosa of the mouth, where they have long been recognized and their presence designated as Fordyce's disease, has been explained as resulting from aberrant buds along the fetal line of closure.<sup>8,9</sup> This explanation cannot be accepted for their occurrence in the parotid and submaxillary glands, because there is no line of closure in those glands and because there is definite proof that these sebaceous glands are derived from duct epithelium, which seems to have the ability to give origin to them. This ability probably can best be explained on the basis of a latent potentiality of differentiation in the adult epithelium. The existence of this potentiality is likely because, as Gruenwald<sup>10</sup> stated, "latent developmental potencies and lability of determination and differentiation are not, as early investigators believed, peculiarities of embryonic cells." If this latent potentiality of the duct epithelium is accepted, it would be easy to understand its differentiation into sebaceous

glands since both parotid and sebaceous glands are developed from the ectoderm.<sup>11-13</sup> Whether or not this explanation is accepted, it is evident that the duct epithelium can give origin to sebaceous glands in normal parotid glands as well as in those which are the site of neoplasia. Moreover, the latter group seems to have a greater tendency toward such differentiation, as appears to be substantiated (1) by the occurrence of sebaceous glands in greater numbers and in a more advanced state of development in such parotid glands than in those considered to be normal; (2) by the frequency with which sebaceous glands were seen in parotid glands which had given origin to tumors of the muco-epidermoid type, in which it has been observed that the duct epithelium may undergo transformation into mucus-forming cells and also into cells capable of showing different stages of transformation, varying from the normal type cell of the duct epithelium to cornifying squamous elements; and (3) by the occurrence of sebaceous glands in two parotid glands that were the site of pure adenomas, tumors that in themselves may be considered as the direct result of the latent potentiality of the glandular epithelium to undergo differentiation into cells of a new type.<sup>1</sup> It also appears that the epithelium of the parotid gland seems to be more liable to undergo differentiation than that of the submaxillary gland, as is shown by the greater incidence of sebaceous glands in the former. This is further proved by the fact that Hamperl<sup>2</sup> found only 4 instances of sebaceous-gland-like transformation in submaxillary glands in a study of salivary glands in 85 persons, while in the material of the present report this was observed in 28 parotid glands obtained from 51 individuals. From what has been said, it seems logical to think that an epithelium, in which an exaggerated latent potentiality of differentiation exists, is prone to give origin not only to normal structures, but also to tumors in which the cell component may resemble various epithelial elements of ectodermal origin, such as those sometimes found in salivary gland tumors. Thereby are explained some findings which have been noticed repeatedly in the mixed tumors of the salivary glands, such as the presence of squamous epithelium<sup>14-17</sup> and keratohyalin, the resemblance of some salivary gland tumors to the basal cell carcinoma of the skin,<sup>16-20</sup> to the adamantinomas,<sup>20-22</sup> and also to some tumors occurring in relation to the skin but with characteristics similar to mixed salivary gland tumors.<sup>23</sup>

#### SUMMARY

Sections from 100 normal parotid glands and from 133 parotid glands which were the site of tumors were studied to find out (1) if structures resembling sebaceous gland are present in normal parotid glands, (2) if



such structures are present in parotid glands that are the site of tumor, and (3) if the presence of sebaceous glands might have some significance in the explanation of tumors occurring in the salivary glands.

From this study, the following results were obtained. Structures which could not be differentiated from sebaceous glands, measuring from 50 to 350  $\mu$  in diameter, were seen to originate from intercalated and striated ducts which ended blindly. Such sebaceous glands were present in 28 per cent of the normal parotid glands and in 24.8 per cent of the parotid glands in which tumors had occurred. Their occurrence was not related to sex, age, or state of atrophy of the parotid gland. Sebaceous glands were more numerous and in a more advanced state of development in the parotid glands in which tumors had occurred.

Sebaceous glands were associated with 17 mixed tumors, 8 muco-epidermoid tumors, 6 carcinomas, and 2 adenomas. There appears to be a high incidence of sebaceous glands in parotid glands in which muco-epidermoid tumors, carcinomas, and adenomas develop. There seems to be some evidence of greater susceptibility of the parotid epithelium to undergo differentiation as compared with that of the other salivary glands, which gives a possible explanation for the greater frequency of tumors in the parotid glands.

It is reasonable to believe that the sebaceous glands occurring in the submaxillary and parotid salivary glands arise from duct epithelium owing to the presence of latent potentiality of differentiation in that epithelium. This aids in developing a possible explanation for the origin of some of the components of the mixed tumors.

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## DESCRIPTION OF PLATES

All photomicrographs were obtained from slides stained with hematoxylin and eosin, unless otherwise stated.

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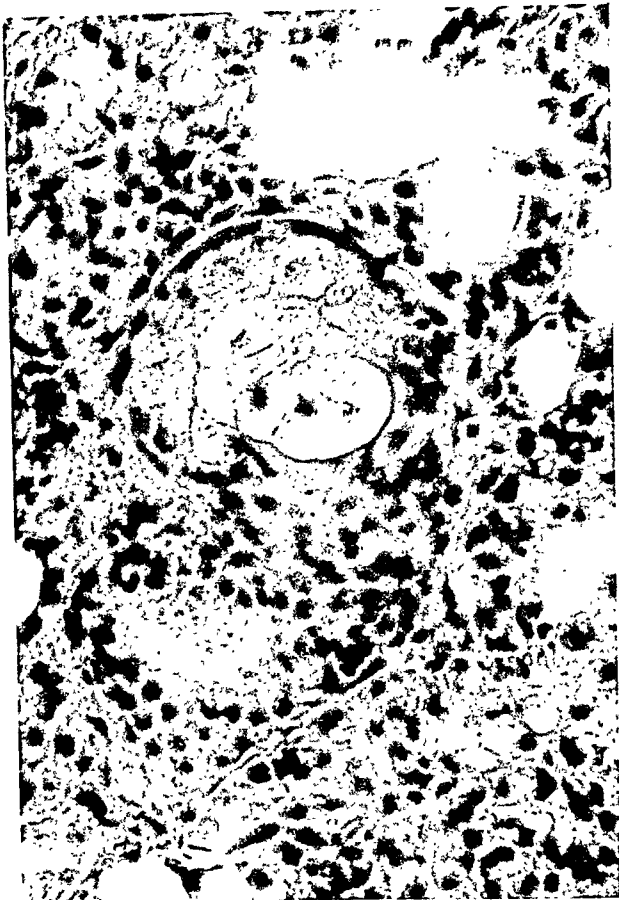
### PLATE 96

- FIG. 1. Case 18 of Table II. Sebaceous gland arising in a branch of a striated duct. Of note is the holocrine secretion falling into the lumen of the duct.  $\times 375$ .
- FIG. 2. Sebaceous gland in the parotid gland of the oldest person from group 1, case 27 of Table II.  $\times 375$ .
- FIG. 3. Sebaceous gland in the youngest individual of group 1, case 16 of Table II.  $\times 375$ .
- FIG. 4. Sebaceous gland in one of the cases of adenoma of the parotid gland (case 12 of Table II). Of note is the holocrine secretion in the lumen of the duct.  $\times 375$ .

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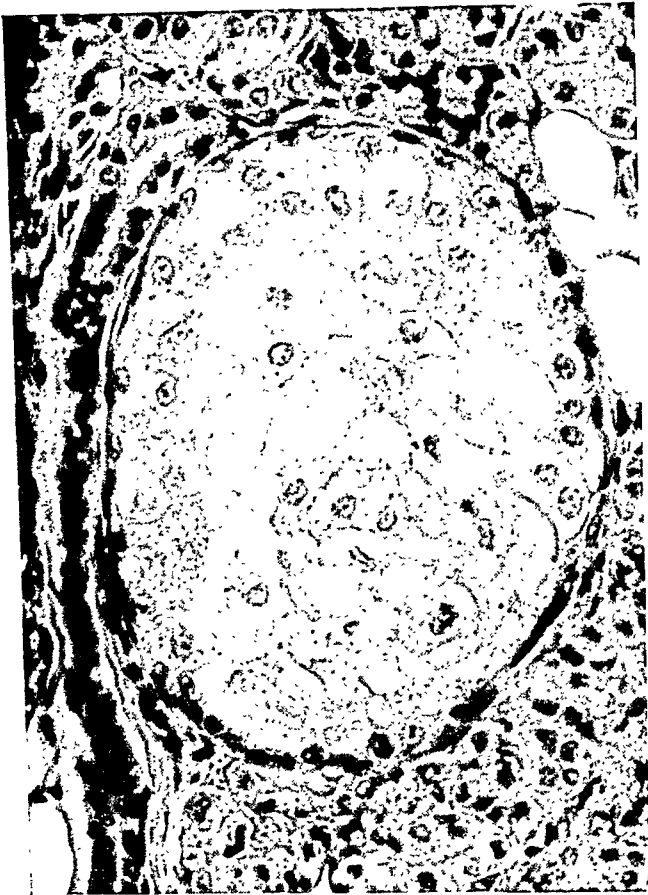
Meza-Chávez

Sebaceous Glands in Parotid Glands

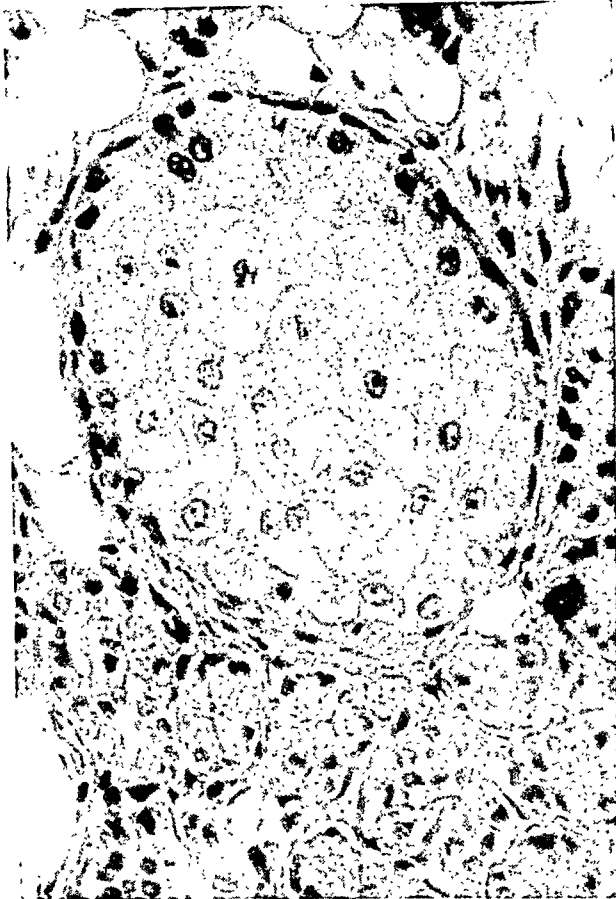
PLATE 97

- FIGS. 5 and 6. Sebaceous glands in a normal parotid gland (case 12 of Table III). Of note is the larger size of the nuclei of the sebaceous glands in comparison to the acinar and ductal cells.  $\times 375$ .
- FIG. 7. Sebaceous gland from another normal parotid gland (case 9 of Table III). There is a mitotic figure in a cell at the periphery of the alveolus.  $\times 375$ .
- FIG. 8. Simple branched sebaceous gland from a case of parotid adenoma, case 12 of Table III.  $\times 375$ .

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Meza-Chávez

Sebaceous Glands in Parotid Glands

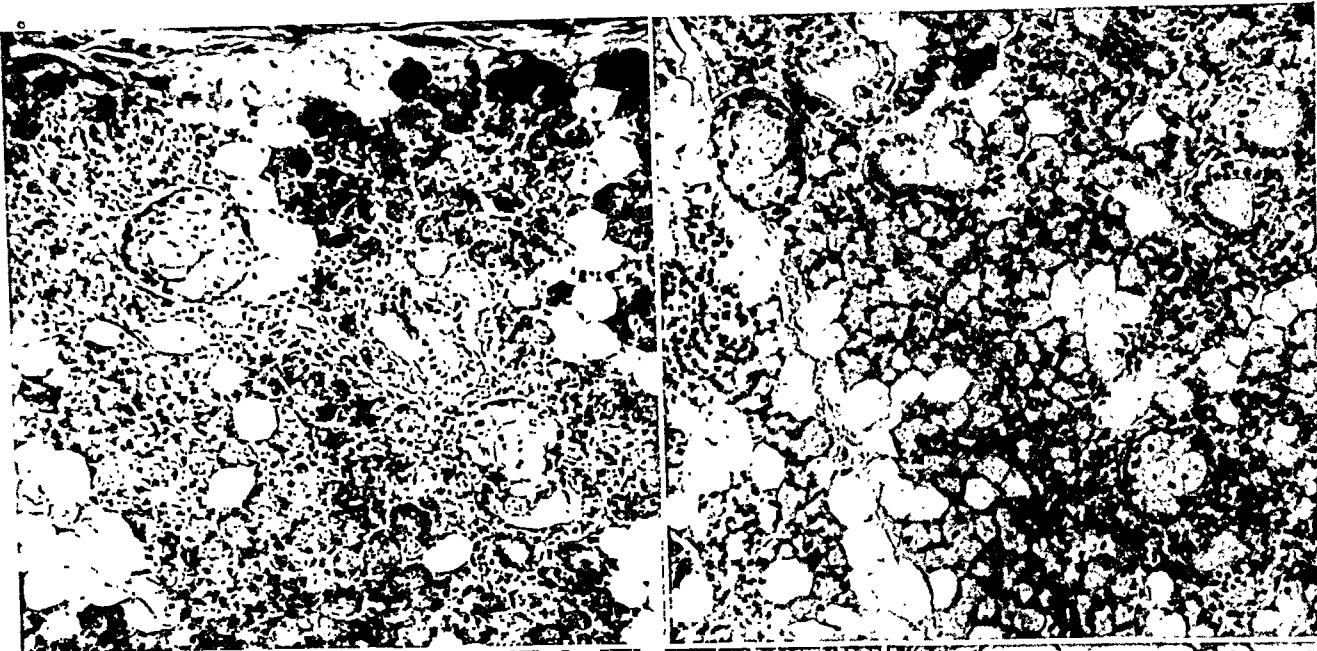
PLATE 98

FIGS. 9 to 13. Parotid glands from group 1 in which two or more sebaceous glands were seen in a single microscopic field (cases 18, 27, 16, 17, and 12, respectively, of Table II).  $\times 115$ .

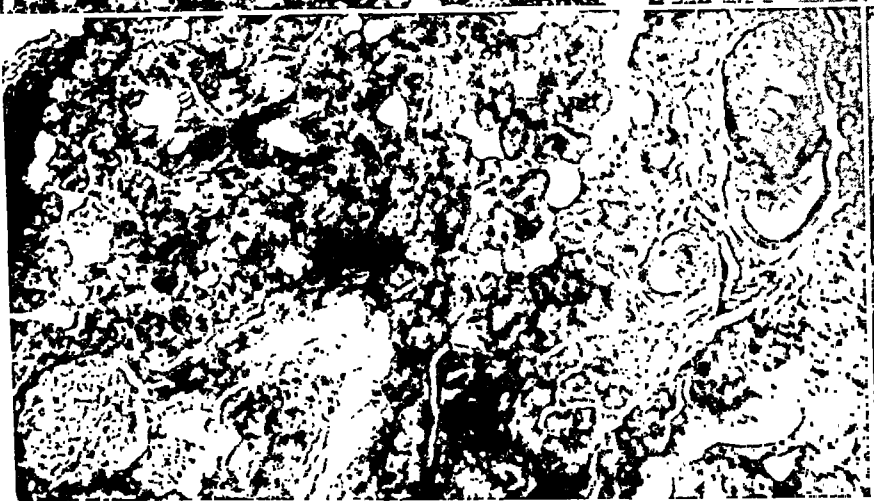
FIG. 14. Parotid gland from group 2 in which two sebaceous glands are seen in one microscopic field (case 7 of Table III).  $\times 115$ .

FIG. 15. Early development of sebaceous glands in a normal parotid gland (case 13 of Table III).  $\times 115$ .

9



11



12



14

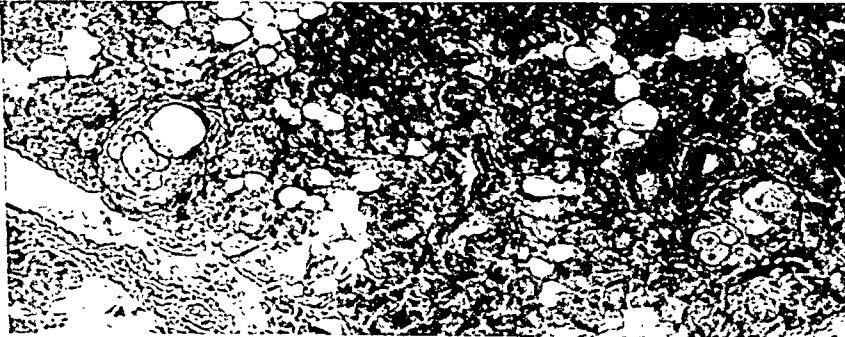




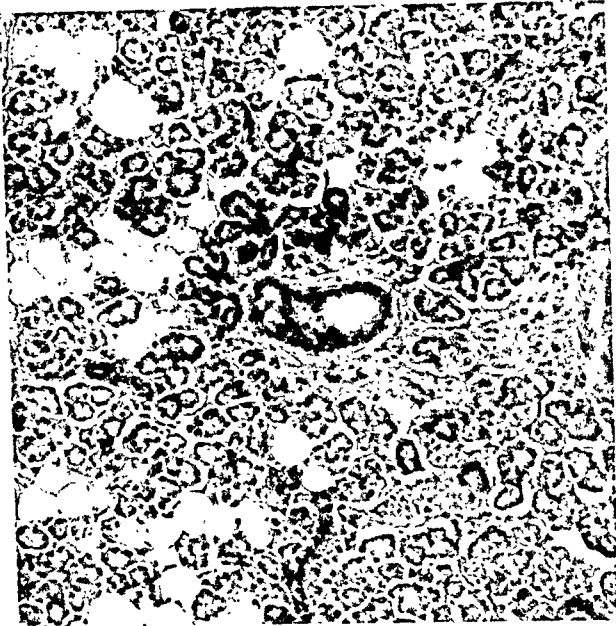
PLATE 99

FIGS. 16 to 18. Three sections from a sebaceous gland arising from an intercalated duct which ends blindly (case 10 of Table III).  $\times 115$ .

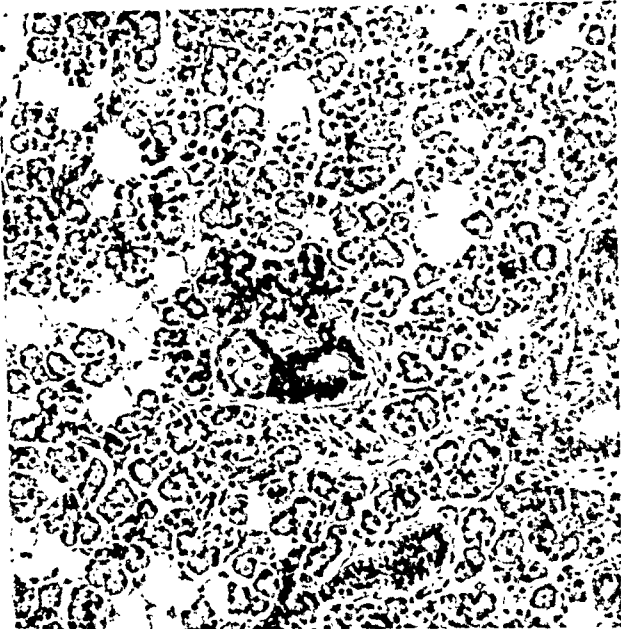
FIGS. 19 and 20. Sebaceous glands from normal parotid glands, with lymphocytic infiltration around them (cases 13 and 15 of Table III).  $\times 115$ .

FIG. 21. Frozen section from a normal parotid gland showing a sebaceous gland (case 9 of Table III). Hortege's silver impregnation and scharlach R stains.  $\times 115$ .

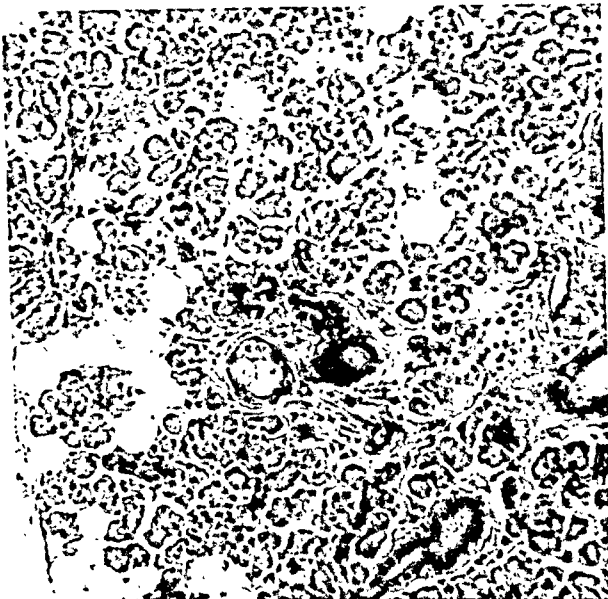
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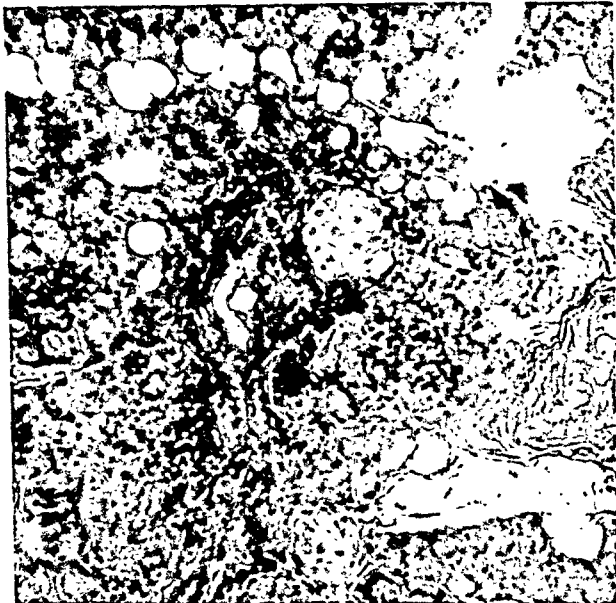
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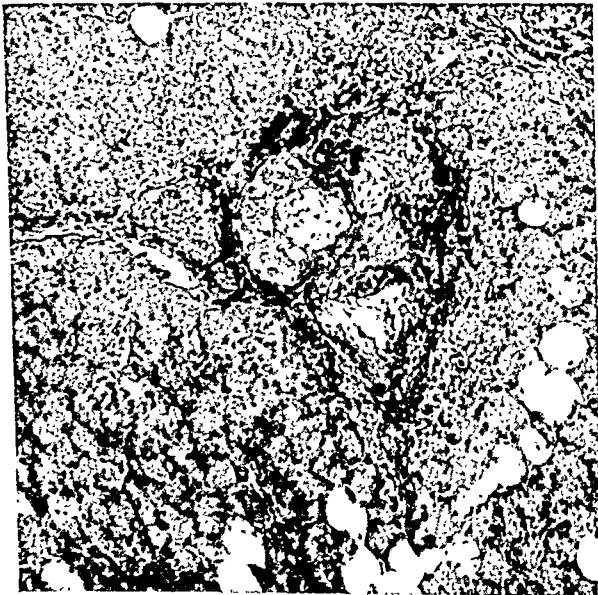
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21



Meza-Chávez

Sebaceous Glands in Parotid Glands



## COMBINED LIVER CELL AND BILE DUCT CARCINOMA\*

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Carcinomas of the liver displaying features indicating origin from both liver cells and bile ducts have been observed infrequently. They have been reported under various terms: mixed tumors,<sup>1,2</sup> duplex type,<sup>3</sup> intermediate type,<sup>3,4</sup> carcinoma of dual origin,<sup>5</sup> cholangiohepatoma.<sup>6</sup> The first case so designated appears to be that of Wells.<sup>7</sup> Others have been recorded by Goldzieher,<sup>8</sup> 1 case; Tramontano and Fittipaldi,<sup>9</sup> 2 cases; Koster and Kasman,<sup>3</sup> 1 case; Rasario,<sup>10,11</sup> 2 cases; Bonne,<sup>4</sup> 1 case; and Cabot case no. 26441.<sup>12</sup> Gustafsen,<sup>5</sup> reporting on 62 cases of primary carcinoma of the liver, mentioned 2 of intermediate type. In the reports of primary liver cell carcinoma by Prescott,<sup>13</sup> Moon,<sup>14</sup> Larson,<sup>15</sup> and Neumann,<sup>16</sup> the data were very suggestive that the bile ducts participated in the malignant process. This communication is a report of 5 additional cases, found among 35 cases of primary carcinomas of the liver in 8175 consecutive autopsies performed at City Hospital, Welfare Island, between May 1, 1911, and May 1, 1948.

Usually a sharp distinction can be drawn between the two principal forms of primary carcinoma of the liver. Microscopically, Winternitz<sup>17</sup> distinguished between them on the bases of cell type, architectural pattern, and stromal reaction. These criteria generally have been employed by subsequent writers. Typically, liver cell carcinoma is comprised of large polygonal cells with a moderate amount of pink granular cytoplasm and large hyperchromatic vesicular nuclei with one or more prominent eosinophilic nucleoli. The cells are arranged in trabeculae along a capillary sinusoidal bed. The resemblance to normal liver tissue may be, and often is, unmistakable. The cells may contain bile. Giant cells are said to be more characteristic of hepatocellular carcinoma, although they occur infrequently. Kupffer cells are likewise said to be a distinguishing feature. In contradistinction, carcinoma derived from bile ducts consists of irregular cuboidal or columnar cells, sometimes showing stratification, with scanty basophilic cytoplasm and small hyperchromatic nuclei. There is usually a large amount of dense connective tissue in which the epithelial cells are arranged in irregular cords and duct-like formations. In appearance they do not differ from scirrhous tubular adenocarcinoma of other organs.

Certain peculiarities, however, serve to confuse one type with the

\* Received for publication, July 29, 1948.

other, and in carcinoma of double nature, these features must be evaluated with even greater caution. Since the great majority of liver carcinomas occur with cirrhosis, the structural features of this condition must be carefully borne in mind. As noted by Hoyne and Kernohan,<sup>18</sup> the condensation of bile ducts and connective tissue in cirrhotic livers accompanying primary liver cell carcinoma should not be interpreted as a mixed neoplasm. It has been our experience also that this proliferation is sometimes so intense that it approaches a true adenoma and may mimic neoplasia, particularly if an inflammatory cellular reaction is absent. Nodular hyperplasia of the liver cells may be so marked that it can be distinguished from true neoplasia only with difficulty. Ewing,<sup>2</sup> Cunningham,<sup>19</sup> Winternitz,<sup>17</sup> and many others have emphasized the fact that liver cell carcinomas may produce structural features resembling bile ducts. In addition, degenerative and metaplastic changes may alter the appearance of the tumor so as to make its positive identification difficult. Thus, central necrosis of columns of carcinomatous liver cells may simulate bile ducts.

Since the majority of primary carcinomas arise in cirrhotic livers and may be unicentric or multicentric in origin, three combinations of tumors of double nature are possible. (1) Separate neoplastic masses may be comprised entirely of the liver cell type on the one hand, and of the bile duct type on the other. (2) Contiguous masses, each of different character, may mingle as they grow. (3) Individual masses may display both features so intimately associated that they can be interpreted only as arising from the same site. Each of these possibilities is illustrated by authentic cases in the literature and in our material.

Separate nodules of hepatocellular and bile duct character were present in case 1 (autopsy 3542). The liver, weighing 3600 gm., was studded with hard white masses and did not appear cirrhotic. The white masses consisted of characteristic bile duct carcinoma—ducts lined by malignant cells lying in abundant dense fibrous tissue. This was the predominant finding. The cirrhotic process in the remainder of the liver was mild and many nodules had characteristic malignant liver cells with the sinusoidal bed preserved. Metastases of ductal character were present in the hilar and upper abdominal lymphatics. A thrombus showing a mixture of neoplastic ductal and hepatic cells was present in a hepatic vein; pulmonary metastases were similar.

Contiguous carcinomatous nodules of different character were present in case 2 (autopsy 5305). The liver, which weighed 5150 gm., was extremely cirrhotic and studded with soft yellow, and hard white,

masses. The nodules of hepatic cell type predominated and varied considerably in appearance. Some had the familiar cords of large granular cells separated by a sinusoidal bed. In others the cells were extremely anaplastic, small, and mimicked cells of lymphoid nature. The bile duct nests were very scirrhous. Although the two types were frequently contiguous, there was no distinct intermingling. The metastases, of bile duct nature, were confined to the hilar and upper abdominal nodes; there were no metastases of liver cell type.

The malignant types were in more intimate association in case 3 (autopsy 254). The liver was enlarged, cirrhotic, and had many masses of variable size and appearance. Most of the nodules were of liver cell character, Kupffer cells were abundant, and both hepatic and portal venous radicles had similar tumor thrombi. One nodule (Figs. 1 and 2) had groups of large cuboidal cells with opaque cytoplasm at its periphery, in sharp contrast to the adjacent malignant hepatic cells which had a lighter, finely granular cytoplasm containing fine bile pigment. Near by, within the fibrous tissue of the portal area, were ductal structures lined chiefly with large cuboidal opaque cells, intermingled with somewhat larger cells having more abundant, finely granular cytoplasm, similar to the cells deep in the nodule. There appeared to be transitions between the two kinds of cells. Bile canaliculi could not be demonstrated. Metastases were absent.

Extraordinary intermingling of the carcinomas occurred in the remaining two cases. The liver in case 4 (autopsy 5490) was extremely cirrhotic; weighed 1150 gm., and had a soft yellowish brown mass 1.5 cm. in diameter in the right lobe. Microscopically, this mass consisted of nodules containing carcinomatous liver cell cords and bile ducts (Fig. 3) separated by thin-walled capillaries. The cords had large cells with finely granular, faintly acidophilic cytoplasm. Bile canaliculi were not seen. The ducts had large opaque basophilic cells. An occasional duct was lined by cells of both types (Fig. 4), each distinct, sometimes adjacent, and apparently without transitional forms. The nodules were free of fibrosis. One small nodule of pure liver cell type was present. There were no metastases.

The liver of case 5 (autopsy 7262) weighed 4200 gm., was intensely cirrhotic, and had a variety of colors in the nodules—yellow, gray, green, tan, and white. Most of the nodules were similar to those of case 4, showing an intimate mingling of malignant ducts and liver cell cords. In some areas where duct formation was more prominent, extremely fine fibrosis was present. Transitional forms between the cell types was evi-

dent, especially where they lined ductal structures. Some nodules showed a single malignant type, either liver cell or bile duct. One carcinomatous duct showed marked papillation. The metastases were of mixed nature and were present in the aortic, tracheobronchial, and right renal lymph nodes and in the right adrenal gland.

The views concerning the specificity of liver and bile duct cells are at once controversial and contradictory. Embryologically, it is well established that liver cells and bile ducts have a common origin from the primitive gut.<sup>20</sup> For the normal liver, McIndoe<sup>21</sup> and others have described a gradual transition from the cuboidal epithelium of the bile ducts to the large liver cells, and the presence of an intermediate epithelial type. Rindfleisch<sup>22</sup> believed that cells of bile ducts may originate from the parenchymal cells. On the other hand, in regenerating liver associated with cirrhosis, MacCallum<sup>23</sup> saw what he believed to be new liver cells arising from bile duct epithelium. Muir<sup>24</sup> likewise believed that in certain cases bile duct epithelium may be transformed into hepatic cells. Lucké<sup>25</sup> observed the differentiation of hepatic cells from biliary epithelium. That either cell gives rise to the other has been challenged by Counseller and McIndoe,<sup>26</sup> Fox and Bartels,<sup>27</sup> and Mallory,<sup>28</sup> who contended that each cell type remains specific and may reproduce only its own kind.

There is no less contradiction regarding the interpretation as to histogenesis of the findings in mixed tumors of the liver. Ewing<sup>29</sup> held that most neoplasms originate from adult cells and that the anaplasia of tumors bears no relationship to embryonal reversion. He expressed the opinion<sup>2</sup> that the mixed tumor had either a dual origin or had possibly arisen from cells which earlier assumed the characteristics of pseudo-bile ducts. Counseller and McIndoe<sup>26</sup> believed that each cell type gives rise to its corresponding tumor entity, and never to the other. Wells<sup>7</sup> believed that the tumor he reported had origin from both liver cells and bile ducts. Goldzieher<sup>8</sup> considered that undifferentiated liver cells may assume the form of pseudo-bile ducts, from which bile duct carcinoma may arise. Moon<sup>14</sup> noted what he believed to be liver cells originating from proliferating bile ducts in a case of primary liver cell carcinoma. Our own material fails to throw light on this question. In one case of intimate association of the two types of malignant cells, there appeared to be transitional forms. In the other, each cell seemed to remain specific.

#### SUMMARY AND CONCLUSIONS

Five cases of combined primary liver cell and bile duct carcinoma illustrate three combinations of tumors of double nature: (1) separate

nodules of hepatocellular and bile duct carcinoma; (2) contiguity with intermingling; (3) intimate association due to origin from the same focus. The present conflicting views of the relationship of hepatic and ductal cells in the histogenesis of these neoplasms cannot be resolved on the basis of this material.

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#### DESCRIPTION OF PLATES

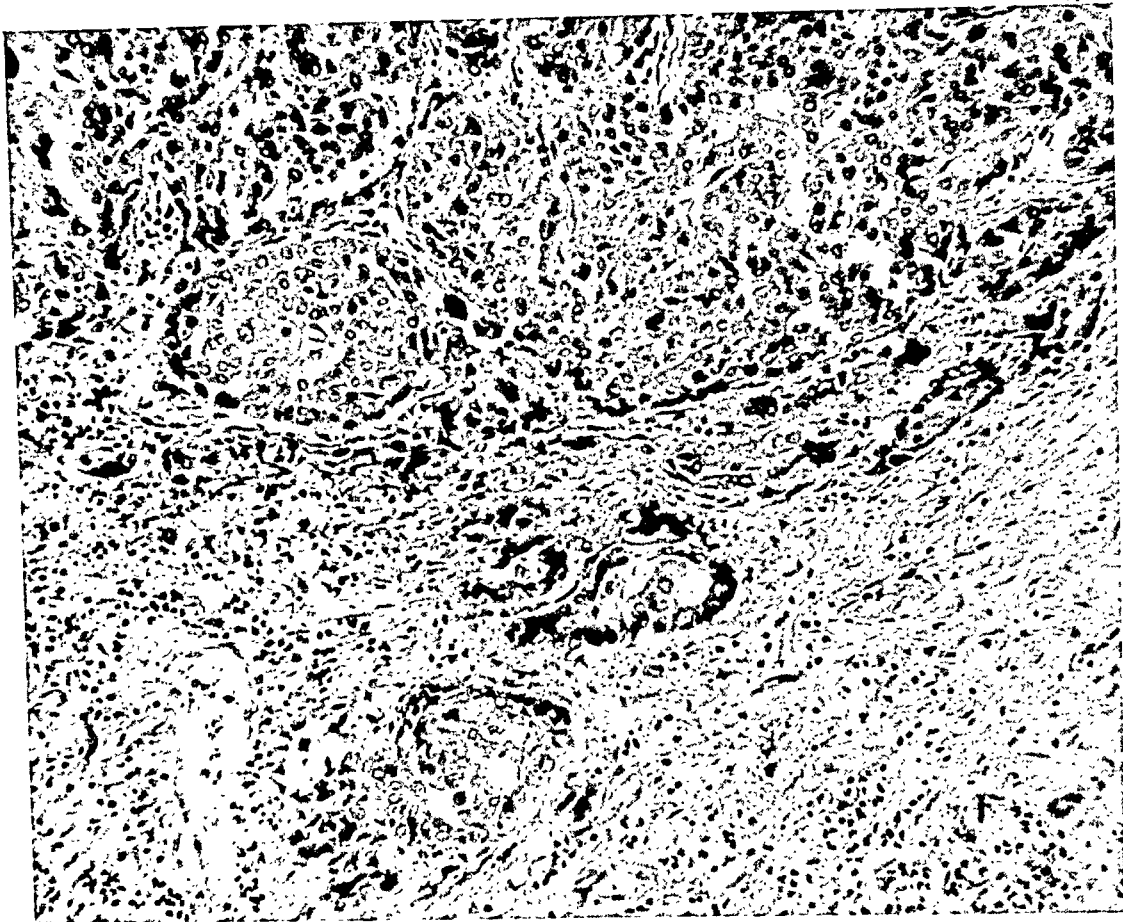
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##### PLATE 100

FIG. 1. Case 3. A nodule showing chiefly a neoplasm of liver cell type. At the periphery, some groups have a zone of more opaque cells suggestive of the bile duct type. In the surrounding fibrous tissue there are ductal structures with bile duct and liver cell epithelium.  $\times 135$ .

FIG. 2. Case 3. A duct is lined partly by large cuboidal opaque cells and partly by large granular cells of hepatic parenchymal character. Cells of the two types apparently have transitional forms.  $\times 400$ .

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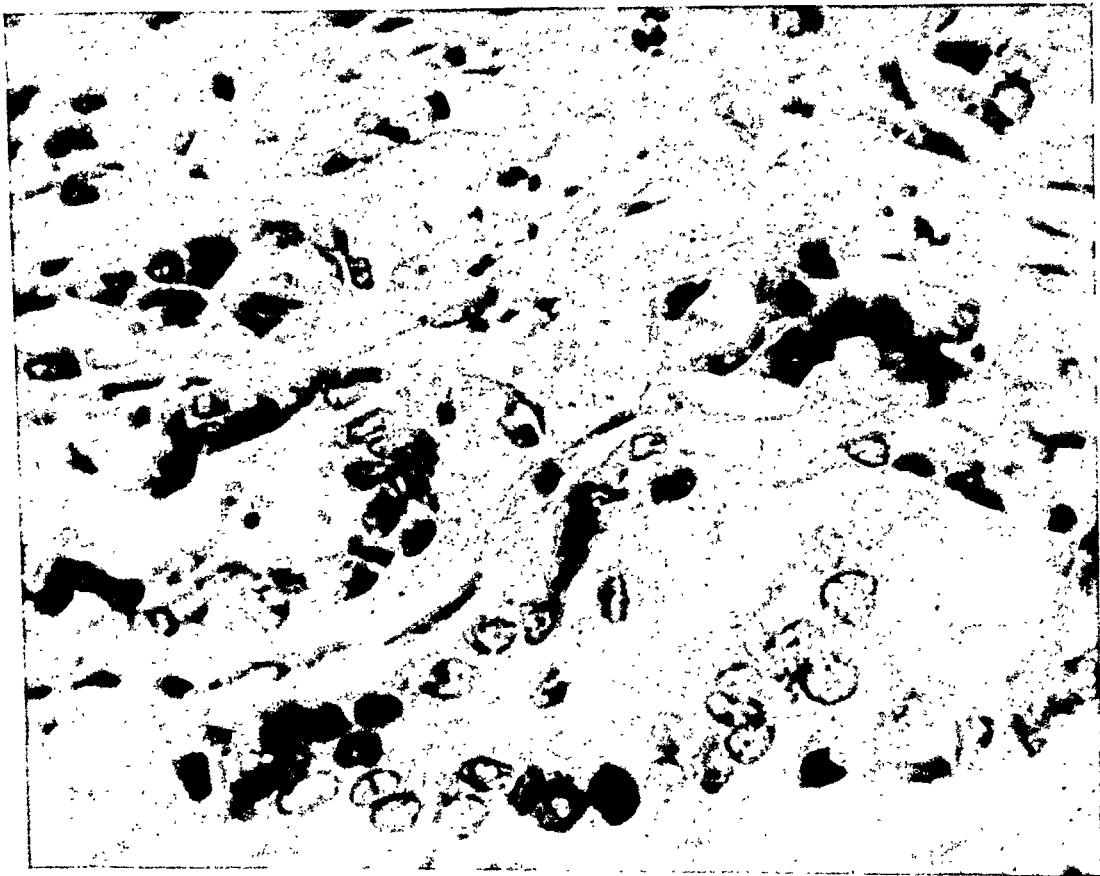
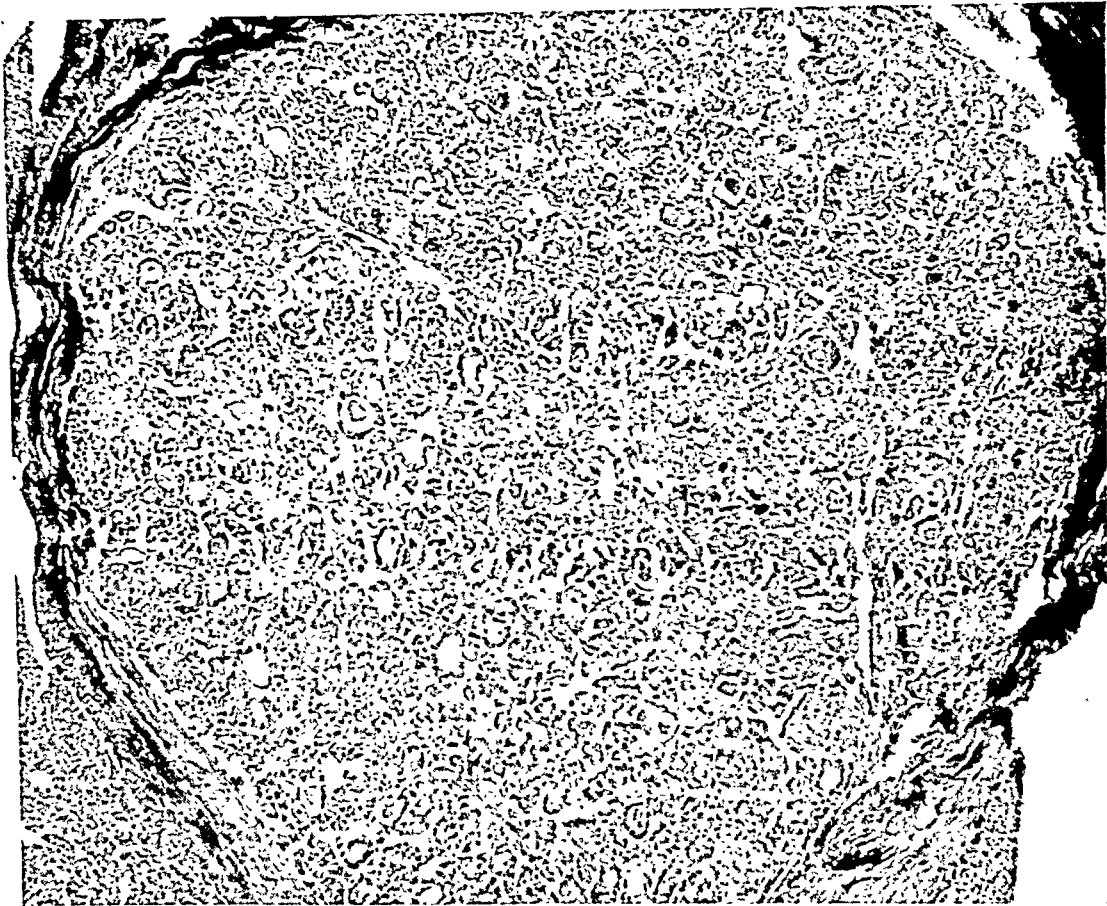


PLATE 101

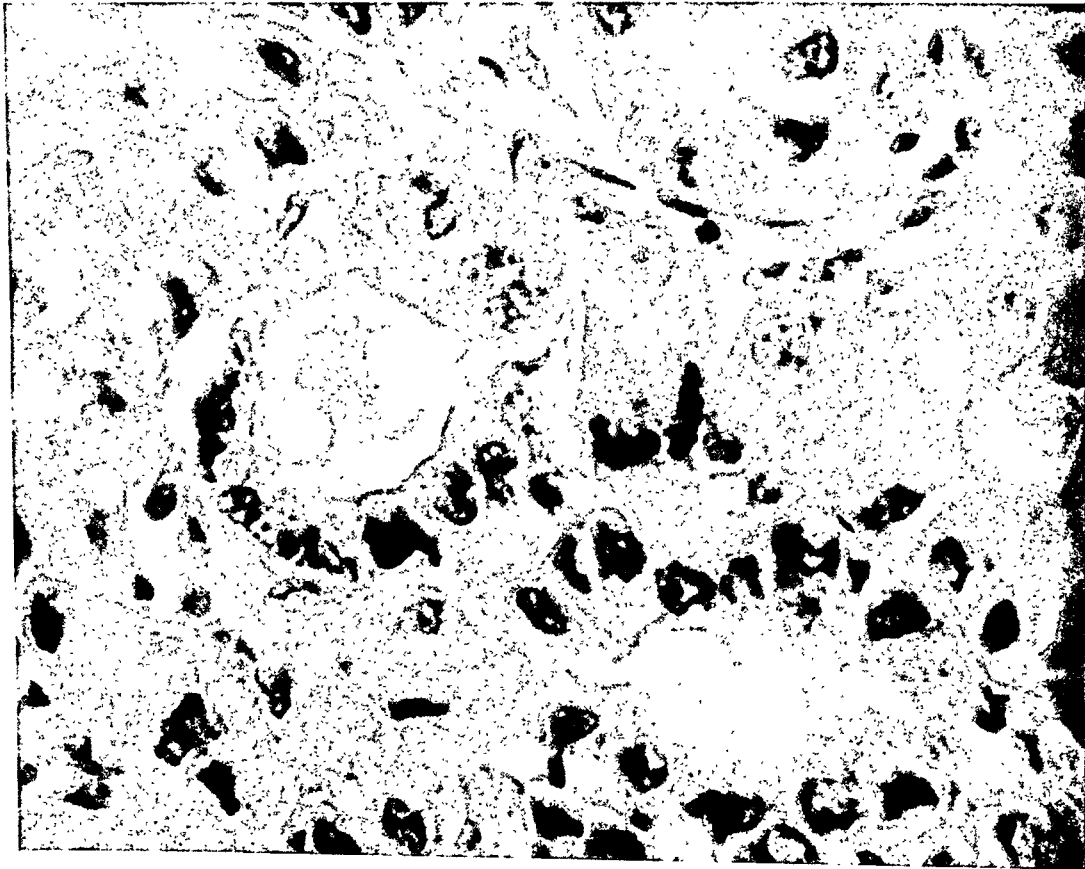
FIG. 3. Case 4. A nodule surrounded by a fibrous wall has an intimate mingling of ducts and cords.  $\times 45$ .

FIG. 4. Case 4. The ducts are lined chiefly by large cuboidal or columnar opaque cells, a more granular cell occasionally being present, as in the duct on the left. Other small nests are composed of large granular cells only, some of giant size.  $\times 400$ .

3



4





## PATHOGENESIS OF ULCERATIVE COLITIS\*

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Ulcerative colitis has been thoroughly reviewed clinically,<sup>1-5</sup> but no pathologic investigation has been made recently, perhaps because the predominating manifestations are those of nonspecific inflammation. The importance of this condition demands that it be better understood. In the hope of advancing the fragmentary knowledge of its evolution, a series of 120 surgically treated and 60 autopsied cases of ulcerative colitis has been reviewed. All autopsies available, and surgical cases from the 20-year period of 1927 to 1946 were included. The series comprised approximately two-thirds of all cases diagnosed as ulcerative colitis in this laboratory. Ulcerative colitis of specific etiology, such as tuberculosis, amebic or bacillary dysentery, lymphopathia venereum, vitamin deficiency, and uremia, was excluded. Cases lacking sufficient histologic material for detailed analysis were discarded.

A clinical classification of nonspecific ulcerative colitis has been provided by Bargaen<sup>1</sup>: type 1, or thrombo-ulcerative colitis, attributed to diplostreptococcal infection; type 2, or regional colitis, affecting mainly the right colon; and type 3, or atypical ulcerative colitis, also termed "idiopathic." These distinctions do not lend themselves to close pathologic correlation. In the course of study of our cases, two distinct pathogenetic processes were observed which lead to ulceration. They are termed type A (vasculitis) and type B (crypt abscess). Half the cases gave no clue as to their pathogenesis and are called indeterminate.

### INCIDENCE

Among chronic intestinal diseases, ulcerative colitis is relatively common.<sup>1,2</sup> Studies have shown either equal sex distribution,<sup>5-7</sup> or a preponderance of females.<sup>8,9</sup> Women made up 55 per cent (100) of the 180 cases being analyzed, and men 45 per cent. The type A (vasculitis) cases, 19 in number, included 15 women, and type B (crypt abscess) cases numbered 71, with 33 women and 38 men. The 90 indeterminate cases included 52 women and 38 men.

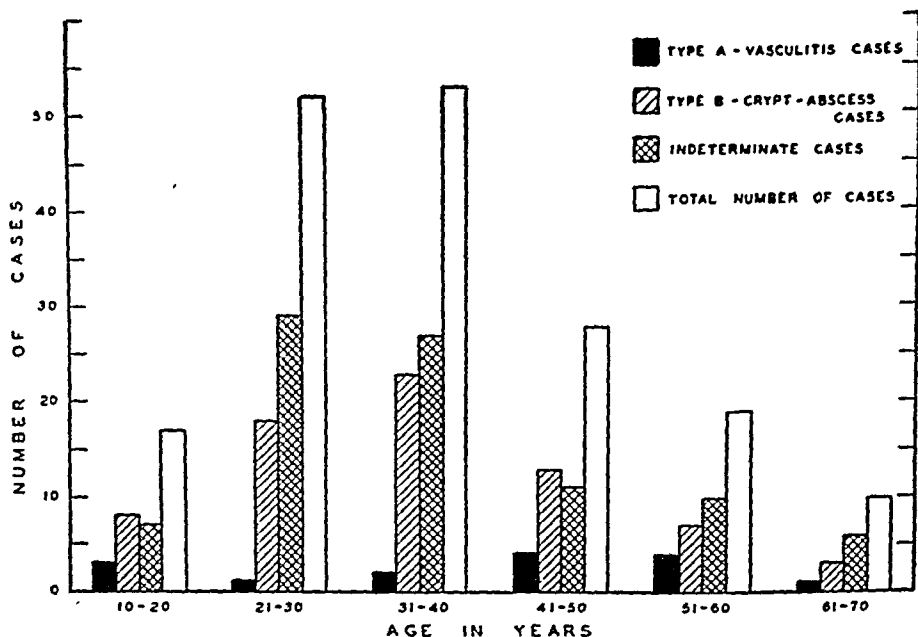
Ulcerative colitis is reported to occur largely in the twenties and thirties.<sup>6,9</sup> In the present series, 104 cases (58 per cent) fell in these decades, but the age range was 11 to 67 years. Cases in the newborn,<sup>10,11</sup> childhood,<sup>12,13</sup> and old age<sup>14</sup> also have been recorded. There were no significant fluctuations in the age incidence of type A (vasculitis) cases,

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as shown in Text-Figure 1. Type B (crypt abscess) cases showed a peak incidence in the third and fourth decades, similar to that of cicatrizing enteritis. No additional significant information was provided by combining the analyses of sex and age.

### SYMPTOMS AND COURSE

Diarrhea with blood and mucus, abdominal cramps, and tenesmus are the most important symptoms. Fever, weakness, tachycardia, malnutrition, and mental abnormalities often are prominent. Ulcerative colitis may have either a sudden or insidious onset,<sup>15,16</sup> and may pursue a fulminating,<sup>17</sup> slowly progressive, or irregular course with exacerbations



Text-Figure 1. Age distribution by decades of total number of cases and of the cases of each type of ulcerative colitis.

and remissions for many years.<sup>6,8,15</sup> Thirty years ago, 50 per cent of the patients died,<sup>15</sup> and collected series of over 1200 more recent cases<sup>6,7,9,18,19</sup> have an average mortality of 17.3 per cent. A few patients are cured, and the remainder are affected intermittently for life.<sup>20</sup> Clinically, ulcerative colitis tends to be a chronic irreversible disease, varying in extent, activity, and severity.<sup>18,21,22</sup>

Among our 60 fatal cases, a definite statement of the duration of ulcerative colitis was made in 54. Seven patients lived 2 months or less after onset, 12 lived from 2 to 6 months, and 3 lived from 7 to 12 months. Thus 22 patients (37 per cent) had histories of 1 year or less. The disease lasted from 1 to 5 years in 19 cases (32 per cent). There were 7 cases of 5 to 10 years' duration, 5 cases with 10 to 20 years of colitis, and 1 patient succumbed after 27 years. Symptoms of more than 5 years' duration

were present in 13 (22 per cent). Equal numbers of type A (vasculitis) and type B (crypt abscess) patients died within 1 year of onset.

### DISTRIBUTION

The extent and location of the lesions of ulcerative colitis are interesting. Clinically, the ulcers are said usually to begin and become most widespread in the rectum and sigmoid.<sup>7,15,23,24</sup> Accessibility to examination may contribute to overemphasis of this early ulcerative proctitis. It is often said that thereafter the ulcers gradually spread upward until

TABLE I  
*Distribution of Ulcers in Intestine, and Accompanying Conditions*

Cases	Ileum	Colon					
		Cecum	Ascending	Transverse	Descending	Sigmoid	Rectum
45 autopsy	21	28	30	37	39	43	37
64 surgical	16	39	49	50	51	47	45
109 total	37(34%)	67(61%)	79(72%)	87(80%)	90(83%)	90(83%)	82(75%)

Segmental distribution:		2 cases
Right colon only:		3 cases

<i>Appendix</i>			
Present 61 cases	Appendicitis:	Acute	1 case
		Recurrent acute or healing	4 cases
Absent 6		Ulcerated or chronic	12 cases
		Healed	10 cases
Negative 11 cases		Healed with partial obliteration	7 cases
		Healed with obliteration	8 cases

<i>Accompanying Conditions</i>	
Mucosal polyp	8 cases
Multiple mucosal polyps	2 cases
Melanosis coli	3 cases
Meckel's diverticulum	4 cases
Other diverticula	3 cases
Calcified mesenteric lymph nodes	3 cases

the whole colon is involved. The right colon is ulcerated first in about 10 per cent of cases, and later the lower colon.<sup>19,23</sup> However, there is some doubt about these statements.<sup>5,25</sup> It may be that the colon usually is affected by the disease simultaneously, in part or as a whole, without subsequent spread.

Adequate data as to the distribution of the lesions were available in 109 of our 180 cases. The observations from autopsies are probably of greater value, since the surgical material tends to be influenced by clinical and technical factors. In 45 autopsies, the sigmoid was most often ulcerated, the descending colon next most frequently, and the cecum least commonly. No difference was found in the distribution of lesions in cases of the vasculitis type and of the crypt abscess type. The incidences are given in Table I. Extension of ulcerative colitis to the ileum in one-



third of the cases is about the frequency previously reported.<sup>26</sup> Ulcerative colitis clearly involved the appendix in one-fifth of our cases, and possibly affected it in another three-fifths. This is one of the exceptional diseases accompanied by chronic appendicitis.

Twenty-five female patients with total colectomies had aggregate lengths of large intestine averaging 86 cm., with a range of 56 to 116.5 cm. Thirty similar male cases had an average colon length of 99 cm., with extremes of 74 and 140 cm. The normal length of the large intestine is said to be approximately 150 cm.<sup>27</sup> In ulcerative colitis, as a result of contraction of scar tissue, the colon is thus shortened by about one-third.

Accompanying conditions, such as melanosis coli, Meckel's or other diverticula, calcified mesenteric lymph nodes, and mucosal polyps are listed in Table I. The incidences are comparable with those in the general population.<sup>28-30</sup> Neoplastic polyps were distinguished from inflammatory pseudopolyps by pathologic criteria described elsewhere.<sup>31-33</sup>

#### GROSS APPEARANCE

The dramatic picture of severe ulcerative colitis viewed from the mucosal surface is familiar to most physicians. The lining of the colon is deep red or purple due to marked hyperemia and congestion, and is wet and shining with blood and mucus. Petechial hemorrhages are scattered over the surface. The ulcers vary in number, size, shape, extent, and distribution. The smallest are punctate, and at times pus may be expressed from them; or ulcers may be so broad and coalescent that only a few irregularly shaped, elevated epithelial patches remain, and the mucosa is practically absent from part or all of the colon (Fig. 1). More frequently there are narrow, longitudinal, gutter-like depressions, three or four in number, extending through most of the length of the colon. These may overlie the muscular taeniae,<sup>34</sup> but are usually between them. A network of girdling ulcers connects the longitudinal gutters. Characteristically the submucosa is exposed but not penetrated, and its surface is shiny, red or gray. Soft, bulbous, edematous pseudopolypoid nodules are formed by the remaining mucosa, and these overhang and may entirely conceal the ulcers. Stretching or fixation of the colon will expose concealed ulcerations (Fig. 2).

During active progression of ulcerative colitis, the wall of the colon is irregularly thickened. Among 73 cases in which the maximum thickness was recorded, it averaged 0.6 cm. The range was 0.1 to 2.0 cm. Ulcers at times penetrate the muscle layers, usually in several places, and only subserosal connective tissue contains the fecal stream. In consistency the intestine then becomes as friable as wet paper.

A marked contrast is provided by the relatively unchanged external appearance of the unopened colon. The serosa is slightly edematous and congested. The mesentery contains scanty fat and numerous enlarged soft lymph nodes. The bowel lacks the normal sacculations and is slightly contracted, which is attributable to thickening of the submucosal and muscular layers.<sup>8</sup>

Roentgenologic study by barium enema gives additional information. In the early stage a marked spasm of the rectal ampulla is found,<sup>35</sup> which, if overcome, permits rapid filling of a negative-appearing colon. Later the mucosa presents a feathery pattern due to ulcerations and edema. Eventually, pseudopolyps may become recognizable. The tendency is for the colon to become narrowed and shortened, forming a stiff tube-like channel.<sup>36</sup> Margins of the diseased area are rarely sharply demarcated or segmental as in cicatrizing enteritis.

Proctoscopic observations reported by Buie<sup>37</sup> and Bargaen<sup>1</sup> allow four stages of the developing disease to be distinguished: (1) hyperemia, (2) edema, (3) multiple, minute, superficial abscesses, and (4) coalescence with formation of large ulcers. Occasionally one may see (5) mucosal regeneration and healing.

The colon in a late stage of ulcerative colitis, when healing has followed medical treatment or surgical treatment such as ileostomy, appears quite different. The lumen is uniformly narrowed, with real or apparent overgrowth of mesenteric fat, which surrounds almost the entire outer surface. The circumference in 49 cases measured averaged 3.4 cm., with extreme values of 1.0 and 8.0 cm. This would indicate a lumen averaging 1.1 cm. in diameter. The mucosa is thin, with smooth and granular patches. It is usually tan or yellow with gray lymphoid aggregates, scattered pseudopolypoid tags, and punctate or longitudinal pitted and creased scars representing sites of healed ulcers. The bowel wall is indurated by submucosal fibrosis and muscular thickening. The serosa is slightly stiffened and opaque. Regional lymph nodes are small.

Gross distinctions can be made between the cases of type A (vasculitis) and type B (crypt abscess). Cases of type A are characterized by more severe, more extensive, and deeper ulcerations. Much of the thickness of the colonic wall is affected locally by a process similar to infarction elsewhere in the body. This is correlated with a higher mortality, since 12 of the 19 cases of this type form part of the autopsy group. Only 5 of the 19 developed pseudopolyps, which are an indication of long-continued superficial damage. Cases of the crypt abscess type correspond to the most common type of ulcerative colitis, as described grossly by Bargaen<sup>1</sup> and others, and proctoscopically by Buie.<sup>37</sup> Pus can at times be ex-

pressed from superficial mucosal abscesses. Pseudopolyps were present in 49 of 71 cases. The mortality of colitis of this type is apparently less, since only 18 of the 71 cases are in the autopsy group. Among the 90 indeterminate cases, 9 were pathologically inactive, and 27 had pseudopolyps. The indeterminate group comprises exactly one-half in each instance of the total number of surgical cases and of the autopsy cases. Histologic observations form the basis of this classification.

### MICROSCOPIC FINDINGS

Changes in the mucosa and submucosa of ulcerative colitis are most important histologically. Epithelial continuity is interrupted by ulcers which tend to be multiple and shallow. Beneath the ulcers is a heavy infiltrate of lymphocytes, plasma cells, eosinophils, and macrophages, with very vascular granulation tissue. Inflammation may remain localized above the muscularis mucosae, but usually the inner half of the congested, edematous submucosa is involved also. Foci of perivascular and perineural chronic inflammation are observed in the outer submucosa, intermuscular septa, and subserosa. Pathologically, little is gained by separating ulcerative colitis into acute and chronic stages. Chronic inflammatory exudate, granulation tissue, and fibrosis are present very soon after the onset, and conversely, after years of the disease, foci of acute inflammation may still be found.

Type A (vasculitis) cases were so classified because of lesions of blood vessels, such as necrosis of the vascular walls, thrombosis, arteritis, phlebitis, periarteritis, or several of these abnormalities. Medium-sized submucosal arteries were affected uniformly, and in early stages the overlying mucosa appeared unchanged (Fig. 3). Subserosal or mesenteric vessels sometimes were similarly damaged. Thrombosis usually was present and the appearance often was identical with periarteritis nodosa or thrombo-angiitis obliterans except for the location in the intestine (Figs. 4 and 5). Interference with the local blood supply led to sloughing of part of the mucosa and damage to the wall beneath, which in the most severe cases resulted in gangrene and multiple perforations of the intestine. In later stages, hyaline vascular sclerosis with obliteration or recanalization of some large vessels was observed. Vasculitis was found in 19 cases (11 per cent) of our series, 15 being women, and 12 patients died. Bargen, in his microscopic studies of "thrombo-ulcerative colitis," described similar changes,<sup>1</sup> but he ascribed them to a more common, less severe variety of ulcerative colitis, clinically and grossly closely resembling the next type to be considered.

Type B (crypt abscess) colitis was initiated by the appearance of

polymorphonuclear leukocytes within the lumina of individual mucosal crypts just above their bases. A little eosinophilic mucoid secretion sometimes was mixed with the leukocytes or layered along the epithelium (Fig. 6). No abnormality of the epithelium was seen. As more polymorphonuclear leukocytes accumulated in an affected crypt, the base was distended, with flattening of the epithelium (Fig. 7). Eventually the crypt abscess ruptured at the lower end, spilling its contents into the submucosa. Purulent material dissected beneath the adjacent epithelium, which was ulcerated away (Fig. 8). Pus then escaped into the lumen of the bowel. Occasionally an abscess persisted in the submucosa, topped by a single layer of viable epithelium. Mucosal bridges were formed beneath which the purulent exudate flowed (Fig. 9). This undermining inflammation predisposed to the development of overhanging mucosal islands at the ulcer margins. In time, edema and chronic inflammation of the remaining mucosa produced localized pseudopolypoid projections. The lesions described were superficial and irritated the deeper layers of the colon without seriously damaging them. Fresh crypt abscesses appeared to occur in crops for a long time, periodically reactivating the partially healed colitis. Abscess formation was not encountered in the connective tissue of the lamina propria or submucosa except after rupture of crypt abscesses, and no abnormalities of the lymphoid follicles, which play an important rôle in bacillary dysentery,<sup>3</sup> were observed. Seventy-one cases (39 per cent) of this series were of crypt abscess type, and 18 cases were fatal.

In 3 necropsies vasculitis and crypt abscesses were observed together.

Focal granulomas were present in 11 of the 71 crypt abscess cases, either in the colon or regional lymph nodes. They resembled the tubercles of sarcoid, and frequently contained giant cells. Sometimes a granuloma originated from the site of a crypt abscess, but granulomas did not accompany vascular lesions. Seven of these cases were clinically and pathologically indistinguishable otherwise from ulcerative colitis, and the granulomas were considered to be incidental secondary manifestations, such as are met with in some examples of carcinoma of the stomach or breast. The remaining 4 cases were controversial, since diagnoses of cicatrizing enteritis (regional ileitis) extending to the colon might be entertained.<sup>38</sup> All of them had involvement of the small intestine, affecting up to 68 cm. of ileum. Concomitantly, the entire length of the colon showed changes grossly and microscopically compatible with ulcerative colitis. Probably both diseases were present.

An indeterminate microscopic appearance was found in 90 cases (50 per cent). The pathologic changes either were so extensive or of such

long duration that it was impossible to decide how they developed. Thirty of these cases were fatal. Lymphoid follicles with germinal centers were prominent in the submucosa of 20 indeterminate, 19 crypt abscess, and 3 vasculitis cases. They were considered to result from long-continued ulceration and secondary bacterial infection. Pseudopolyps were present in 28 of the indeterminate cases (Fig. 10). By means of multiple sections and special attention to a search for early lesions, it would probably be possible in future studies to reduce the number of indeterminate cases. The indeterminate group may include lesions of types as yet undescribed, as well as vasculitis and crypt abscess cases.

Intestinal changes secondary to ulcerative colitis comprised, from the lumen outward, mucosal regeneration, hypertrophy of the muscularis mucosae, submucosal fibrosis and fatty infiltration, muscular hypertrophy, and subserosal fibrosis (Fig. 11). Epithelium at first covered the healing surface to a variable extent with a single layer of cuboidal cells, which later formed primitive glands but rarely reproduced the orderly, well differentiated appearance of normal mucosa. The muscularis mucosae often became markedly thickened. The submucosa was densely scarred and infiltrated with fat. Muscular hypertrophy, particularly of the circular layer, was the rule. Mild fibrosis was found in the subserosal connective tissue. The regional lymph nodes, at first edematous and hyperplastic, eventually developed fibrous thickening of their capsules.

#### COMPLICATIONS

One of the distressing features of ulcerative colitis is the number and variety of complications, attributable to inflammation, lowered resistance, malnutrition, and unknown processes. The incidence of complications in this series is given in Table II and compared with clinical series previously reported.<sup>6,7,30-41</sup> Perforation of the colon or ileum, either spontaneously or postoperatively, is the most lethal. Either a local abscess or generalized peritonitis may result, and the latter is more common. Other acute events are ileal intussusception and perirectal abscesses, which rupture and form fistulas in ano. Later complications following scarring of the colon include stricture or even obliteration of a portion of the lumen. Previous ileostomy encourages cicatricial contraction, but is not a prerequisite.

Carcinoma arising in ulcerative colitis has attracted considerable and deserved attention.<sup>42-44</sup> Nine cases (5 per cent) of this series developed carcinoma of the colon, frequently of high-grade malignancy, and in 2 cases carcinoma arose in multiple sites. The average age of these patients was 41 years, with duration of ulcerative colitis from 3 to 13 years.

Carcinoma develops in ulcerative colitis by two processes. First, neoplastic mucosal polyps are present in patients with ulcerative colitis to the same degree as in the general population, and have the same tendency to become malignant, accelerated, perhaps, by chronic inflammation. Second, regeneration of mucosal epithelium continues despite repeated ulcerations, and eventually foci of precancerous epithelial hyperplasia develop in the unfavorable environment. Such areas were observed in 4 cases in this series, with an average duration of 10 years of ulcerative colitis. Inflammatory pseudopolyps, because of their structure, are considered least likely to undergo neoplastic transformation, and no convincing case of this has been found.

TABLE II  
*Complications of Ulcerative Colitis*

	Present series 180 cases	Collected from literature 2029 cases
<i>Intestinal</i>		
Perforation	15 (8%)	41 (2.0%)
Ileum 5		
Colon 10		
Peritonitis	27 (15%)	
Abscess or fistula	11 (6%)	95 (4.7%)
Stricture	3 (2%)	134 (6.6%)
Carcinoma	9 (5%)	37 (1.8%)
Multiple primary sites	2 (1%)	
<i>Nutritional</i>		
Emaciation	12 (7%)	
Anasarca	5 (3%)	26 (1.3%)
Fatty liver	33 (18%)	
<i>Cause undetermined</i>		
Thrombosis	12 (7%)	26 (1.3%)
Pancreatic dystrophy	45 (25%)	
Liver necrosis	6 (3%)	
Liver cirrhosis	3 (2%)	
Nephritis, all types	17 (9%)	35 (1.7%)
Arthritis	5 (3%)	93 (4.6%)
Skin disease	16 (9%)	77 (3.8%)
Endocarditis	3 (2%)	17 (0.8%)
Myocarditis	3 (2%)	
Amyloidosis	3 (2%)	
Adrenal necrosis	1	

Apart from the intestinal tract, thrombosis was the most common, relatively acute complication. It occurred in two-fifths of the autopsied cases, most frequently in the iliac veins, but occasionally elsewhere, as in the jugular veins, aorta, and mesenteric arteries (Fig. 12). Mural cardiac thrombi were present in 3 cases. Generalized thrombophlebitis and arterial thromboses in ulcerative colitis are serious rare complications.<sup>45,46</sup>

A type of pancreatic dystrophy, recently investigated,<sup>47</sup> was found in three-fourths of the 60 autopsies. It consists of focal dilatation of terminal ducts and related acini, which are filled with plugs of eosinophil

secretion, at times layered. Some acinar cells in affected areas show metaplastic eosinophilic cytoplasm (Fig. 13). The appearance strongly suggests local stagnation of exocrine secretion, not due to mechanical obstruction or other abnormality of larger ducts. Cause of the dystrophy is unknown. One suggestion is secretin deficiency.<sup>47</sup> In a control series of 100 autopsies selected to include diseases thought most likely to show this condition, 41 per cent had the same lesions. The pancreatic dystrophy thus appears to be nonspecific.

Malnutrition is a usual sequel to ulcerative colitis.<sup>48</sup> Emaciation results in many cases. Associated anemia<sup>49</sup> and lowered plasma protein are frequent,<sup>50</sup> and anasarca may ensue. Fatty liver, probably also a by-product of malnutrition,<sup>51</sup> was found in 33 of 60 autopsies. Focal hepatic necrosis was observed in 6 cases (Fig. 14). Three other patients had hepatic cirrhosis at autopsy.<sup>52</sup> In two the condition was considered to be toxic cirrhosis, with perilobular chronic inflammation, fibrosis, and disorganization of normal liver structure (Fig. 15). The third showed an early stage of cirrhosis arising in a fatty liver.

Renal disease, usually pyelonephritis, was found in almost 10 per cent of this series. The frequency of skin diseases, particularly focal necrosis, with ulcerative colitis is already well known.<sup>53,54</sup> Arthritis is often present, and the Marie-Strümpell type may be encountered more frequently than might be expected.<sup>55</sup> Toxic myocarditis and amyloidosis<sup>56</sup> were each present in 3 cases. Some rarer complications reported, such as combined systemic disease,<sup>57</sup> hypertrophic osteo-arthritis,<sup>17,58</sup> infantilism,<sup>59,60</sup> and calcinosis<sup>61</sup> were not encountered.

TABLE III  
*Coincidental Diseases in 60 Autopsied Cases*

Cholelithiasis	8
Diabetes mellitus	3
Obesity	3
Peptic ulcer	3
Rheumatic heart disease	3
Myocardial infarct	2
Carcinoma of breast, cervix, gallbladder, and kidney	1 each

Coincidental diseases found with ulcerative colitis are listed in Table III. Four patients had had surgical operations or trauma which might have been factors in precipitating ulcerative colitis.

#### CAUSES OF DEATH

The immediate causes of death in the 60 cases upon which autopsies were performed are tabulated in Table IV, with 41 autopsy reports from other sources.<sup>5,7,10,11,17,46,56,59</sup> In the present series, peritonitis was re-

sponsible for 21 deaths; pulmonary diseases exclusive of embolism for 8; and renal failure and sepsis, for 7 each. The 5 cases of pulmonary

TABLE IV  
*Causes of Death*

	60 autopsied cases, present series	41 autopsied cases, from literature	Total 101 cases
Peritonitis (postoperative, 12)	21	21	42
Emaciation	3	12	15
Bronchopneumonia	2	10	12
Sepsis or toxemia	7	3	10
Renal insufficiency	7	3	10
Thrombosis	2	5	7
Pulmonary embolus	5	1	6
Hemorrhage or anemia	4	0	4
Ileus	2	1	3
Cardiac insufficiency	0	3	3
Lung abscess	2	0	2
Pulmonary edema	2	0	2
Pulmonary tuberculosis	1	1	2
Hepatic insufficiency	1	1	2
Empyema	1	0	1
Undetermined	5	1	6
Multiple causes	5	24	29

embolus correspond to the expected incidence.<sup>62</sup> Gastro-intestinal or intraperitoneal hemorrhage, massive<sup>63</sup> or insidious, caused 4 deaths.

#### ETIOLOGY AND PATHOGENESIS

Continued interest in specific bacterial agents in ulcerative colitis has produced many interesting studies but no convincing results. Bargaen's diplostreptococcus<sup>1</sup> and the *Bacterium necrophorum* of Dragstedt, Dack, and Kirsner<sup>64</sup> are at present regarded as secondary invaders.<sup>65-67</sup> The possible rôle of viruses has not yet been exhaustively investigated. Felsen's thesis<sup>3</sup> that both cicatrizing enteritis and ulcerative colitis result from bacillary dysentery lacks sufficient bacteriologic and morphologic proof, and was not supported by the microscopic findings in the present series. However, dysentery may be one of several trigger mechanisms setting off an ulcerative process.<sup>67</sup>

Psychogenic factors as a background for ulcerative colitis have received general attention.<sup>5,68-70</sup> Personality disorders such as excessive dependency, immature emotional reactions, and over-conscientiousness have been emphasized, as well as prolonged emotional tension. Psychoses are rarely associated with colitis, but mental abnormalities of less severe type are encountered frequently enough to be noticed by many physicians, and ulcerative colitis is sometimes a psychosomatic disease.<sup>71</sup> It is the mechanism by which emotional stress leads to ulceration which is of particular interest to the pathologist. The ingenious experiments



of Lium and Porter<sup>72,73</sup> indicate that spasm of the colon can produce ulceration. But in our series little support was found for their idea that the ulcers lie in relation to taenial muscle bands. Hypertrophic changes predominate in the circular muscle and muscularis mucosae, not in the taeniae. Spasticity of the colon is a common condition not associated with ulceration, and in ulcerative colitis muscular hypertrophy results from the spastic contraction secondary to mucosal ulceration. The muscular thickening is an effect and not the cause of ulceration.

The chief manifestations of ulcerative colitis are seen in and just beneath the mucosa. The gross appearance of the colon often suggests the presence of a destructive substance in the fecal stream,<sup>74</sup> and this impression is strengthened by the improvement in symptoms and in sigmoidoscopic appearance of the mucosa following ileostomy.<sup>75,76</sup> Analysis of feces from patients with ulcerative colitis has shown abnormal quantities of proteolytic enzymes in some cases.<sup>77,78</sup> It is possible that intestinal hypermotility has introduced gastric and small-intestinal enzymes into the colon,<sup>68,79</sup> although increased peristalsis is present in a minority of cases of ulcerative colitis.<sup>80</sup> Evidence that bile or pancreatic juice is the responsible agent has not been forthcoming. Patients with cholecystocolic fistulas only rarely suffer from diarrhea.<sup>81</sup> Animal experiments along similar lines have produced negative results, and patients with ulcerative colitis excrete less trypsin from ileostomy openings than patients with carcinoma.<sup>82</sup>

Colonic mucosa has excretory as well as absorptive functions, and the formation of crypt abscesses suggests that some substance is being secreted or excreted into the affected crypts.<sup>74</sup> No abscesses or other lesions are found in the connective tissue or vessels, which implies that the substance does not become a leukotactic irritant until altered by a change of pH or otherwise after its discharge into the crypt lumina. Release of an enzyme of proteolytic type in the bases of the crypts is an attractive idea which remains unproved. It is of interest that similar lesions occur in the development of chronic gastritis.<sup>83</sup> Three of our cases had analogous abnormal mucosal crypts in the esophagus, stomach, or small intestine, or in combinations of these organs. The crypts contained laminated eosinophilic mucoid material which reacted consistently to several different stains. This material was not regularly leukotactic, and its relationship to ulcerative colitis is uncertain.

## DISCUSSION

Differential diagnosis of colitis of specific etiology has been well discussed elsewhere.<sup>1,2</sup> Chronic bacillary dysentery and ulcerative colitis have given rise to most controversy. Authorities are about equally

divided as to whether they are pathologically distinct<sup>1,7,8,66,67,74</sup> or indistinguishable.<sup>3,6,24,84-86</sup> Our experience inclines us to believe that the histologic characteristics differ. Ash and Spitz<sup>87</sup> described subepithelial mucosal cysts in chronic dysentery, and, in the acute stage, superficial mucosal inflammation and diphtheritic membrane formation. These changes are not observed in ulcerative colitis, although the early stages of the indeterminate group were, of course, not made out. In most discussions of the differentiation of chronic dysentery and ulcerative colitis, insufficient evidence is presented to allow one to draw definite conclusions, and the subject remains mainly a collection of opinions unsupported by published observations. No claim of specificity is advanced for the early lesions described in our cases of ulcerative colitis. Vasculitis may accompany bacillary dysentery, mesenteric thrombosis, or volvulus. Crypt abscesses have been found in control cases of amebic dysentery, lymphopathia venereum, and in one case of generalized vitamin deficiency. All these conditions may at times have similar lesions leading to ulceration.

Cicatrizing enteritis is occasionally very difficult to differentiate from ulcerative colitis.<sup>26,88</sup> This is partly due to inadequate knowledge of the etiology of both diseases, and partly to their coexistence in occasional patients.<sup>89</sup> The 4 cases in this series in which both diagnoses were made emphasize that, since the two conditions may affect the same patient, final distinctions are not always necessary. This does not, however, necessarily justify the conclusion that both diseases are part of a single entity. In fact, they usually differ in distribution and in gross and microscopic appearances, as well as clinically. Vasculitis and crypt abscesses have not been observed by us in cicatrizing enteritis. Among 20 recent cases of cicatrizing enteritis, small groups of macrophages and eosinophilic leukocytes were found in the mucosal crypts of 6. Conversely, the extensive granuloma formation of cicatrizing enteritis<sup>38</sup> does not characterize ulcerative colitis. The scattered foci resembling those of sarcoid occasionally found in ulcerative colitis are similar to those which at times accompany other intestinal diseases such as carcinoma, lymphoma, and diverticulitis. However, the problems of pathogenesis involved are still far from final solution.

Vessels affected by vasculitis usually were confined to the colon. That the lesions are produced by a circulating substance, analogous to the development of glomerulonephritis or periarteritis nodosa, seems reasonable. What causes the colon to be predisposed is unknown.

Crypt abscesses were the most common precursors of ulceration. Preceding hyperemic and edematous mucosal stages have been described

during proctoscopy. The unaltered histologic appearance of all but localized portions of the mucosa near the bases of certain crypts, where the epithelium is flattened by polymorphonuclear leukocytes in the lumen, makes it seem unlikely that absorption of water from the feces has produced a destructive concentration of some solute. Presence of early lesions in a uniform location within the crypts, even though scattered along the course of the intestine, suggests instead that the irritant is being released by the mucosa. The possible rôle of such agents as proteolytic enzymes in ulcerative colitis already has aroused much clinical interest, but their origin from the colonic mucosa has not been considered previously and now requires further investigation.

The importance of psychosomatic influences in the genesis of ulcerative colitis cannot be disregarded. It is already known that fear and other emotional stresses can provoke the release of potent circulating chemical agents, including substances with proteolytic properties,<sup>90,91</sup> and some authors have thought that ulcerative colitis resembles the effect of a circulating toxin upon the colon.<sup>8,92</sup> Any connection of proteolytic enzymes with the pathogenesis of ulcerative colitis remains to be established.

#### SUMMARY

A series of 120 surgical and 60 autopsied cases of ulcerative colitis, a nonspecific chronic intestinal disease particularly affecting young adults, was analyzed pathologically. The sigmoid and descending colon were most frequently ulcerated (83 per cent), and the cecum least often (61 per cent). Investigation of ulcer formation indicated that local vasculitis was responsible in 19 cases (11 per cent), and mucosal crypt abscesses in 71 (39 per cent). These types of colitis also differed significantly in sex and age incidences, gross appearance, and mortality. Ninety cases (50 per cent) were of indeterminate origin. Peritonitis, fatty liver, and a pancreatic dystrophy were the most frequent of many complications observed. As to the etiology of lesions leading to ulceration various possibilities have been considered, but the importance of particular factors remains unestablished.

The greater part of the surgical material was from the Surgical Service of the Lahey Clinic, and other surgical cases were from the practices of Drs. Thomas J. Anglem, James M. Bonnar, Jr., Howard M. Clute, Daniel F. Jones, Halsey B. Loder, Charles C. Lund, Leland S. McKittrick, and Irving J. Walker.

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## DESCRIPTION OF PLATES

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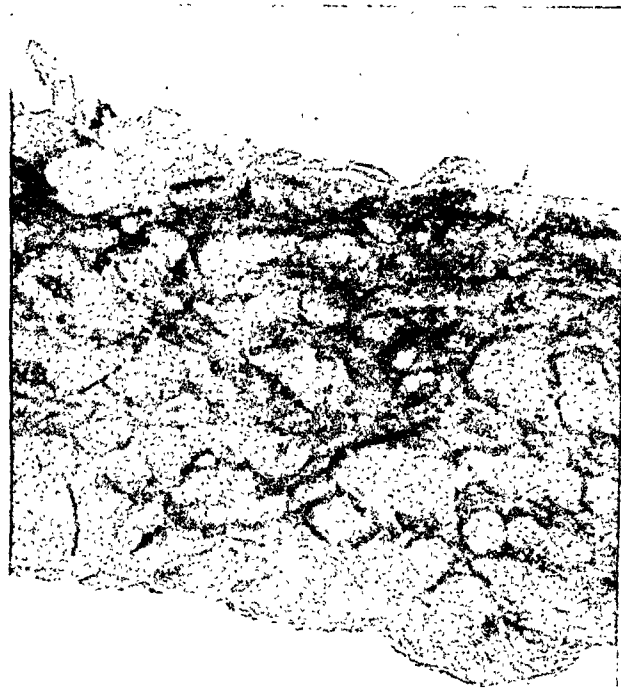
### PLATE 102

- FIG. 1. Mucosal surface of colon, showing localized severe ulcerative colitis in a man, 28 years old. Dark raised islands of persistent or regenerating mucosa are seen in the light-colored region of ulceration. Histologically, the lesions were of indeterminate type.
- FIG. 2. Colonic mucosa showing network of ulcers with pseudopolyps between them, from a case of ulcerative colitis in a woman, 26 years old. The specimen had been fixed in formalin with resulting shrinkage of the pseudopolyps, which in the fresh state concealed the ulcers. Microscopically, crypt abscesses were present.
- FIG. 3. Early vasculitis in the submucosa of the colon. Of note is the marked vascular necrosis with relatively normal appearance of the overlying mucosa. Grossly, extensive ulceration was present. Eosin and methylene blue stain.  $\times 150$ .
- FIG. 4. Vascular necrosis and inflammation in a vessel, larger than that shown in Figure 3, in the wall of the colon. Thrombosis had not yet occurred. Eosin and methylene blue stain.  $\times 150$ .
- FIG. 5. Thrombosis of a submucosal vein in another case which showed vasculitis with ulcerative colitis. Eosin and methylene blue stain.  $\times 125$ .

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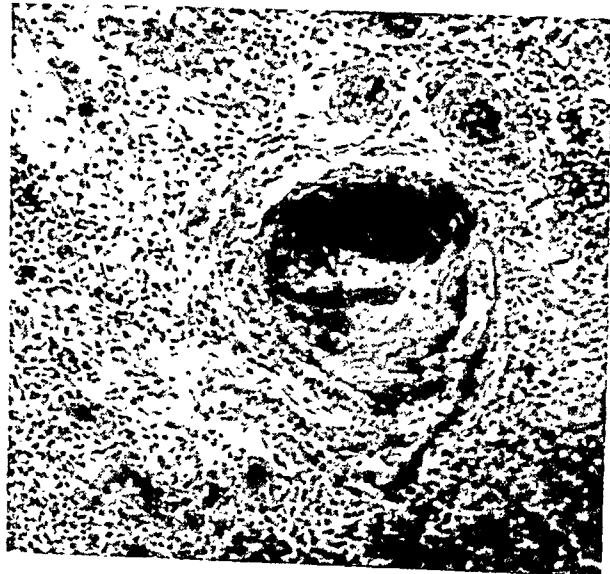
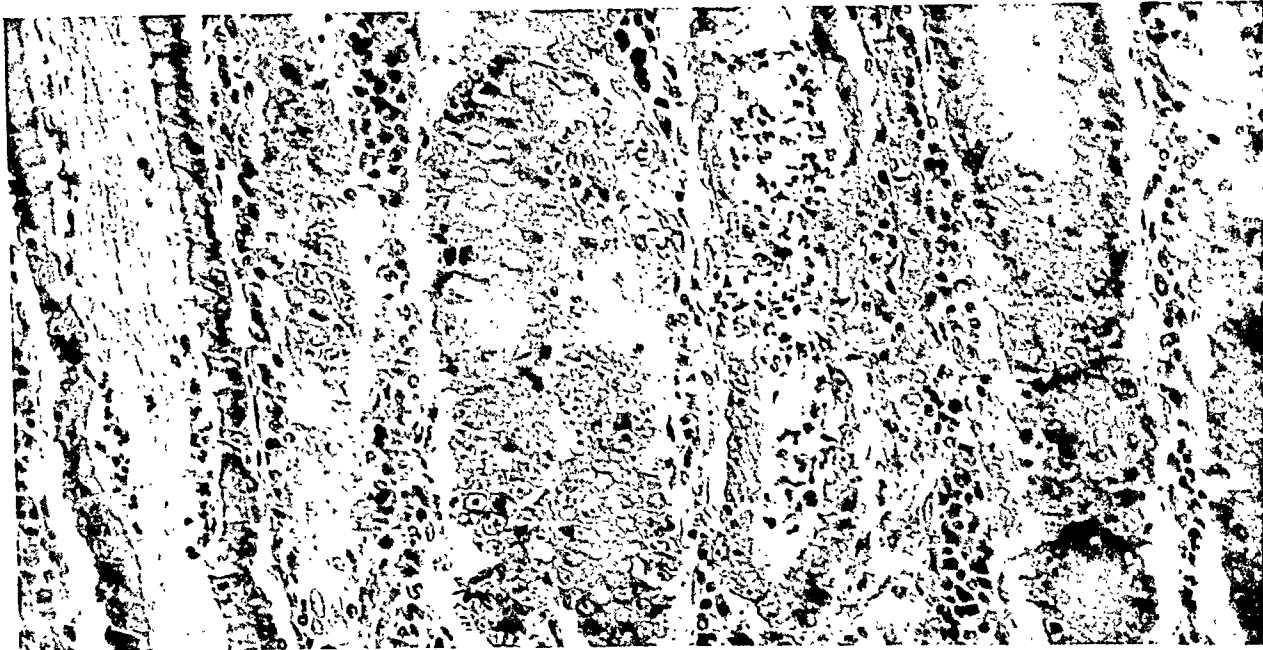




PLATE 103

- FIG. 6. Early stage of crypt abscess formation in ulcerative colitis. Polymorphonuclear leukocytes are found in localized regions near the bases of some crypts. Stringy eosinophilic mucoid secretion is present. The epithelium is not definitely abnormal except where it is compressed, and the lamina propria appears negative. Eosin and methylene blue stain.  $\times 250$ .
- FIG. 7. Well developed crypt abscess compressing and rupturing through the base of a crypt. Eosin and methylene blue stain.  $\times 50$ .
- FIG. 8. Two ruptured crypt abscesses. The mucosa between would have sloughed with formation of an ulcer, although it still appears normal. Eosin and methylene blue stain.  $\times 50$ .
- FIG. 9. Mucosal bridge in ulcerative colitis, beneath which are two ruptured crypt abscesses topped by epithelium. Circular muscle is at the bottom of the picture. Eosin and methylene blue stain.  $\times 25$ .
- FIG. 10. Part of a pseudopolyp. Lymphoid follicles are shown in the connective tissue, and there is epithelial regeneration. Hypertrophy of the circular muscle is also pictured. Phosphotungstic acid-hematoxylin stain.  $\times 10$ .

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PLATE 104

- FIG. 11. A late stage of ulcerative colitis of indeterminate type. From the mucosal surface downward may be seen regenerated epithelium, chronic inflammation of the upper submucosa, fatty infiltration with marked fibrosis of the lower submucosa, and hypertrophy of the circular muscle. All of the major secondary intestinal changes are present except hypertrophy of the muscularis mucosae. Phosphotungstic acid-hematoxylin stain.  $\times 32$ .
- FIG. 12. Organizing thrombus in the inferior mesenteric artery of a fatal case of ulcerative colitis with generalized arterial and venous thrombi. Eosin and methylene blue stain.  $\times 10$ .
- FIG. 13. Pancreatic dystrophy in ulcerative colitis, with dilatation of some terminal ducts and acini. Eosin and methylene blue stain.  $\times 250$ .
- FIG. 14. Focal hepatic necrosis in a fatal case of ulcerative colitis. Eosin and methylene blue stain.  $\times 250$ .
- FIG. 15. Liver showing toxic cirrhosis, with marked inflammation, some fibrosis, and liver cell destruction and regeneration. The liver architecture is destroyed. Eosin and methylene blue stain.  $\times 250$ .

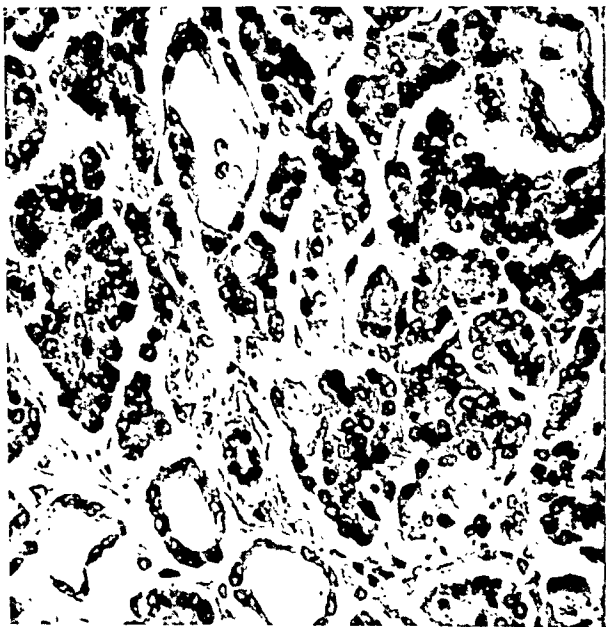
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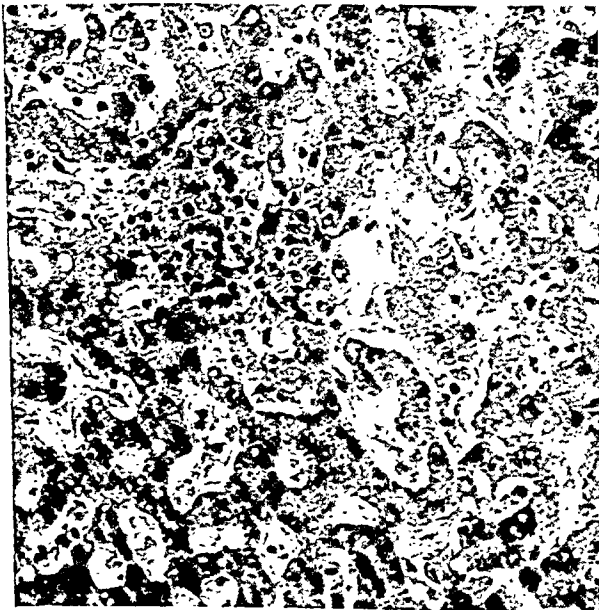
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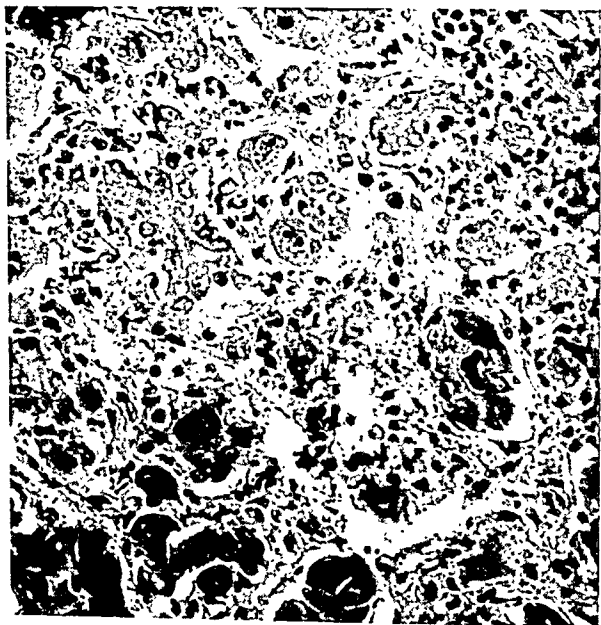
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## NONENCAPSULATED ADRENAL CORTICAL TISSUE IN THE PERI-ADRENAL FAT\*

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In routine autopsy sections at this hospital, adrenal cortical tissue frequently has been observed in the surrounding fat outside the fibrous tissue capsule of the gland. The well known cortical nodules lying adjacent to the gland also at times show perforation of their capsules, with cortical tissue extending into the fat. Such extension of tissue beyond the boundaries of the organ does not fit into the accepted patterns of normal histology, yet this problem has attracted little attention from anatomists or pathologists.

A thorough review of the older literature failed to reveal any mention of the subject (Henle,<sup>1</sup> Huschke,<sup>2</sup> Kölliker,<sup>3</sup> Cornil and Ranvier,<sup>4</sup> Testut,<sup>5</sup> and Soulié<sup>6</sup>). In later years the encapsulated cortical nodules have been much discussed (Broster and Vines<sup>7</sup> and Gruenwald<sup>8</sup>). Kovács<sup>9</sup> described the infiltration of peri-adrenal fat by groups of cortical cells in Addison's disease, differentiating between these nonencapsulated masses and true accessory nodules. He felt that the former were the result of glandular regeneration. A similar picture was described by Huebschmann<sup>10</sup> and Kaiserling.<sup>11</sup> Sudds<sup>12</sup> mentioned that "nodules, islets of suprarenal cortical cells for the most part with a definite zonal arrangement and surrounded partly or entirely with fibrous tissue, were found in 64 out of 214 cases." Goldzieher<sup>13</sup> described nonencapsulated nodules of cortical tissue proliferating loosely into the adjacent areolar tissue in adrenal glands of cases of pemphigus vulgaris. Plaut,<sup>14</sup> in discussing this paper, stated that the picture of suprarenal cortex growing into the surrounding fat tissue was not a rare occurrence in his experience. He thought that this process was unrelated to any particular disease entity, and was part of the general phenomena that endocrine glands may break through their capsule and grow into the surrounding tissue (for example, pituitary body and thyroid).

Gruenwald,<sup>8</sup> however, believed that "outgrowth from the main gland cannot very well be assumed; it is very doubtful that normal cortical tissue should be capable of this extent of infiltrative growth." Broster and Vines<sup>7</sup> merely stated that "small nodules of cortical cells, usually multiple and completely separated from the main gland, though in close approximation to it, are of extreme frequency and occurred in at least 75% of normal subjects." One cannot deduce how many, if any, were

\* Received for publication, August 9, 1948.

without a capsule. Goormaghtigh<sup>15</sup> made no comment on the subject nor did Rogoff,<sup>16</sup> Zwemer,<sup>17</sup> Maximow and Bloom,<sup>18</sup> Bailey,<sup>19</sup> or Cowdry.<sup>20</sup> Bennett<sup>21</sup> did not observe such tissue in the cat. Curiously enough, Yeakel<sup>22</sup> found *medullary* tissue outside of the capsule in her study of ageing rats. From the photographs, these collections appear to be encapsulated. She made no mention of having observed cortical tissue outside of the adrenal gland.

One gathers from the literature that none of the animal adrenals described showed cortical tissue in the peri-adrenal fat. Whether or not such tissue occurs only in human adrenals is problematic. In all of the animal work, mention is made of meticulous trimming of the peri-adrenal fat.

#### MATERIALS AND METHODS

The adrenal glands of 257 adults and 36 newborn infants were examined. These were obtained from autopsies of the years 1942 to 1947. Routine paraffin sections were stained with hematoxylin and eosin. In some of the cases additional sections or ribbons were cut. Occasionally, sections stained for elastic tissue, or by the van Gieson and Bielschowsky methods were available, but there was no sudan-stained material. Attention was focused solely upon adrenal cortical tissue devoid of a capsule, indisputably lying in the peri-adrenal fat. When sections were torn, so as to make observations doubtful, the case was discarded. The frequently encountered encapsulated cortical nodules were not considered in this study. The number of sections per case varied, ranging from 1 to 28.

#### OBSERVATIONS

Of the 257 cases studied, 72 (28 per cent) showed nonencapsulated adrenal cortical tissue in the peri-adrenal fat, and 185 did not. Of the positive cases, 42 (58 per cent) were males and 30 (42 per cent), females. In the negative group there were 104 (56 per cent) males and 81 (44 per cent) females.

As Table I shows, there was a slight increase in relative incidence in the older age groups. Before 50 years of age 21.4 per cent were positive; in those 50 and older, 30.5 per cent were positive.

In 44 cases (61 per cent), a blood vessel, artery, or vein was found in, or in close proximity to, the extracapsular mass. Study of the entire surface of the gland showed that vessels tended to penetrate at certain well defined points, and the extracapsular masses usually were located at one of these points. It is probable, at least in a majority of cases, that a breakout into the fat took place at weak points in the capsule created

by the passage of a vessel, or possibly a small nerve. For some of the larger masses no blood vessels were found nearby.

TABLE I  
*Distribution of Positive and Negative Findings  
by Sex and Age in Decades*

Age groups	Positive			Negative		
0-9	M-0,	F-0	0	M-1,	F-0	1
10-19	M-0,	F-2	2	M-2,	F-0	2
20-29	M-0,	F-1	1	M-1,	F-8	9
30-39	M-2,	F-2	4	M-8,	F-9	17
40-49	M-4,	F-4	8	M-15,	F-11	26
50-59	M-11,	F-10	21	M-27,	F-22	49
60-69	M-15,	F-7	22	M-36,	F-18	54
70-79	M-8,	F-4	12	M-13,	F-10	23
80-89	M-2,	F-0	2	M-1,	F-3	4

The masses varied in size, from a few short parallel columns of cells to relatively large nodules, one measuring 4 mm. They penetrated the fat in a fan-wise fashion, through a gap in the capsule of varying size, with tongues of tissue thrusting out between the fat cells. The shape of the mass varied from round to ovoid or oblong. Some lay entirely free in the fat, away from the capsule of the gland, while others had their longer border parallel to the capsule. The outer surface might be entirely irregular, or flat with wavy borders. The surrounding fat sometimes showed a round-celled infiltration. This usually could be explained by lesions in the adjacent viscera.

The structural arrangement within these masses of cells was very variable. In a few cases they showed the typical arrangement of the adrenal cortex. The zona glomerulosa was nearest to the capsule, with the zona fasciculata in an intermediate position, and the zona reticularis situated distally in the fat. In a great majority of the sections there was a relatively complete loss of normal architecture, often making identification of the various layers difficult. No variations from the normal cytologic picture were encountered, in spite of the apparently disordered and heterotopic growth. The capillary structure was the same as that within the gland. There was no particular hyperemia, as has been described by other authors for these extracapsular masses.<sup>9</sup> In the few sections available, no elastic tissue fibers were seen. That these masses were directly continuous with the intracapsular cortex was shown by continuity of the reticular fibers through the break in the capsule.

The 72 cases were reviewed to determine their zonal composition. In 25 (34.7 per cent), all of the cells were of the glomerulosa type. Cells



of glomerulosa and fasciculata types comprised 30 (41.7 per cent) of the cases. All three layers were represented in 11 (15.3 per cent) cases. In one case cells of the zona fasciculata only were present, and in another cells of fasciculata and reticularis types were identified. In 4 (0.06 per cent), cell type could not be assigned with any reasonable assurance. In 12 cases (16.7 per cent), cells of the zona reticularis were present. They were identified primarily by the presence of pigment.

The number and size of the nonencapsulated adrenal cortical masses were not related to the presence or size of encapsulated cortical nodules or to other variants in the structure of the adrenal cortex, especially hyperplastic processes.

The adrenals of 36 newborn infants were reviewed. In no case was any nonencapsulated adrenal cortical tissue found in the fat. Great care must be taken in such a study not to mistake fetal fat cells for cells of the adrenal cortex.

Adrenal medulla was neither found free in the fat nor within any of the extracapsular masses.

## DISCUSSION

The close proximity of a blood vessel in 61 per cent of the cases indicates that perforation at a weak point of the capsule represents a major means by which these masses penetrate the surrounding fat. Once having broken into the fat, they are free from the normal restraining influence of the connective tissue framework of the cortex. Consequently, the cells can grow in any fashion whatsoever. Serial sections might provide additional information as to their mode of formation. In some of the largest nodules, no adjacent blood vessel was found. It is possible that general unknown growth factors may have some relation to this bursting of anatomic boundaries. In order to substantiate Kovács'<sup>9</sup> contention that the extracapsular cortical masses in his cases of Addison's disease represent efforts of the gland to regenerate, one would have to study a large number of adrenal glands coming only from such cases. The material at hand is insufficient to make such an analysis.

The number of positive cases was not a matter of chance selection of microscopic sections. Positive findings were equally frequent whether there were 1, 5, 10, or 20 sections per case. For one-half of the positive cases there were only one or two sections per case.

It was thought that the presence of carcinoma might be associated with abnormal growth elsewhere. However, the positive findings in patients with malignant growths was proportional to the number with malignant tumors in the entire series. The incidence of infectious diseases

in the material studied was too low to permit conclusions. The increase of positive cases with advancing age has been pointed out. At present there is no adequate explanation for this fact. There were no examples of endocrine disturbances in this study.

It is now fairly certain that the adult adrenal cortex can be formed by differentiation of the cells of the capsule (Zwemer,<sup>17</sup> Wotton and Zwemer,<sup>23</sup> Zwemer, Wotton, and Norkus,<sup>24</sup> Elias<sup>25</sup>), or by mitotic division at the boundary of the zonae glomerulosa and fasciculata (Hoerr,<sup>26</sup> Gruenwald and Konikov,<sup>27</sup> Gruenwald<sup>8</sup>). The dye injection experiments of Salmon and Zwemer<sup>28</sup> have been vigorously attacked by Calma and Foster,<sup>29</sup> McPhail,<sup>30</sup> and Baxter.<sup>31</sup> The objections of the latter authors are undoubtedly correct. The necessity of the capsule for regrowth of the cortex in transplantation has been established by Turner,<sup>32</sup> Ingle and Higgins,<sup>33</sup> and by Baker and Baillif.<sup>34</sup> Williams<sup>35,36</sup> concluded that regeneration in adrenal cortical grafts begins in the glomerular region, and possibly in the capsule. Blackman<sup>37</sup> thought that the normal post-natal reticular zone originates at an early age from persistent cells of the fetal reticular zone which have failed to undergo involution.

The concept that cell replacement in the adrenal cortex takes place by proliferation of the peripheral zone with the zona reticularis representing a senescent zone had been enunciated by Canalis<sup>38</sup> in 1887, and by Mulon,<sup>39,40</sup> and Celestino Da Costa.<sup>41</sup> Later work described more accurately the mode of incorporation of new cells into the cortex, either by end-to-end junction (Zwemer, Wotton, and Norkus<sup>24</sup>), or by apposition from the capsule (Gruenwald and Konikov<sup>27</sup>). Both methods were observed in the material studied.

The observations of this study are consistent with the double mode of growth of cortical cells once they have penetrated into the peri-adrenal fat tissue, namely, by apposition from the capsule and/or by transformation of glomerulosa cells into fasciculata cells. The evidence is insufficient to indicate which mode is more important. Our material furnishes no evidence for the assumption that undifferentiated connective tissue elements in the peri-adrenal fat may, by differentiation *in situ*, become incorporated into the growing mass. The complete absence of nonencapsulated masses in the peri-adrenal fat of the newborn infant is worthy of note, but at present cannot be explained.

Cells of the zona reticularis, as mentioned, were identified in 12 (16.7 per cent) cases. This finding has no relation to the number of sections examined per case. In 5 of the 12 cases there were 5 or less sections. In only 2 cases was the number of cells commensurate with the size of the mass. In the rest they were isolated and few, identified

chiefly by their pigment. In view of these findings, the supposed life cycle of capsule→glomerulosa→fasciculata→reticularis does not necessarily represent the sequence of events in the extracapsular masses. It is possible that these cells differ functionally from those within the capsule; nothing is known about their life span.

### CONCLUSIONS

In 72 of 257 adult adrenal glands, nonencapsulated adrenal cortical tissue was found free in the peri-adrenal fat. Such collections were not found in 36 newborn infants.

The positive cases increase moderately with advancing age.

There was no correlation with any other clinical or anatomic data.

Cells of the zona reticularis were found in only 12 of the 72 positive cases, suggesting that the usually accepted life cycle of cortical cells may not apply to the extracapsular masses and that the latter may differ functionally from the normal cortex.

I wish to thank Dr. Alfred Plaut for suggesting the subject, and for his painstaking guidance during the preparation of this paper.

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# HISTOPATHOLOGY OF OVARIAN PSEUDOMUCINOUS CYSTADENOMA\*

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A varied terminology, now only of historic value, has been applied for more than a century to the pseudomucinous cystadenoma. The term pseudomucin was originally used to describe the content of the locules, which was thought to differ from mucin. Chemically, both mucin and pseudomucin are composed largely of glycoproteins, and differ only in certain physical characters. The exact nature of the secretion is not of diagnostic importance because the tumor is identified by its gross and microscopic features. The literature has been covered by Selye<sup>1</sup> and need not be reviewed further. Pertinent references are made throughout the paper.

This investigation is a detailed histopathologic study of 120 pseudomucinous cystadenomas, with particular attention to the epithelial component.

## MATERIALS AND METHODS

The material consists of surgical specimens submitted to the Institute of Pathology of the University Hospitals. Only typical tumors with characteristic epithelium are included in the study. Formalin-fixed material was utilized for the most part, but in a few instances fresh specimens were preserved in special fixatives. Each tumor was examined with hematoxylin and eosin preparations. Numerous special stains were employed as will be indicated during the course of this report.

## PATHOLOGIC ANATOMY

There was a wide variation in the size of the tumors in this series. The smallest was made up of only a few microscopic locules, while the largest specimen weighed over 22 kg. The cystadenomas usually ranged in weight from 800 to 1500 gm. In only two instances were they bilateral. Some were pedunculated.

Grossly, the cystadenoma consisted of a variable number of locules surrounded by connective tissue. The tumor might be solid with abundant intervening stroma separating small and more or less uniform locules, as in the parvilocular form discussed by Schiller.<sup>2</sup> In the more common multilocular form the locules were larger, more variable in size, and separated by thin connective tissue septa. Mixed forms also occurred. The confluence of smaller locules might eventually lead to the production

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of a predominantly unilocular cystoma, although careful examination disclosed small locules within the capsule. Any of the forms might be papilliferous. This series did not include examples of cystadenoma of the so-called racemous type, in which the tumor resembles "grapes" supported on connective tissue stalks.

The tumor locules contained viscid, transparent or translucent fluid which in some sites was inspissated and firm. With hemorrhage from ruptured vessels, the fluid might be dark red, brown, or black. In the presence of necrosis the contents were yellow and grumous. With necrosis in combination with cholesterol the color might be green.

### *Capsule*

A well defined connective tissue capsule surrounded the cystadenoma except at the ovarian hilum where the capsule was poorly defined or absent. Connective tissue laminae of varying thickness and density made up the capsule, which might also contain spindle-shaped cells resembling smooth muscle but lacking myofibrils in their cytoplasm. Kuge<sup>3</sup> believed smooth muscle exists in the capsule. In agreement with Doran,<sup>4</sup> dense bundles of elastic fibers were found to occur in the capsule, and in the interlocular septa. Their presence probably was the result of displacement from the medulla of the ovary. While there were wide variations in the vascularity of the capsule, larger vessels usually lay in the mid-portion of the connective tissue zone, and numerous capillaries might exist beneath the locular epithelium. Calcification, ossification, edema, inflammation, degeneration, or necrosis altered the pattern of the connective tissue. In the capsules of smaller tumors there might be compressed ovarian tissue.

The innermost connective tissue lamina was situated immediately outside the basement membrane of the locule, and might be thin, hyalinized, and poorly defined. Infrequently, zones or groups of polyhedral epithelioid cells, with clear cytoplasm containing small droplets of fat as demonstrated with sudan IV, surrounded the basement membrane. Between the cell groups were numerous capillaries. In some sites a poorly defined zone of compressed spindle-shaped cells surrounded the locule. Frequently, fat in the form of small droplets of variable size was detected in these cells. Adjacent stromal cells sometimes contained fat droplets of a much smaller size.

### *Basement Membrane*

Between the epithelium and the underlying stroma there was an argyrophilic membrane of variable width and definition. In the younger locules the continuity of the membrane was maintained, but in the larger

locules of mature tumors it usually was broken at many sites, probably because of the rapid enlargement of the locules. The basement membrane presumably was produced by a condensation of the underlying reticulum, and was poorly defined or absent and without surrounding reticular fibers. Fox<sup>5</sup> was unable to demonstrate a membrana limitans, but Malassez and de Sinéty<sup>6</sup> spoke of the presence of an incomplete sub-epithelial endothelium. The presence or absence of such a structure might well depend on the authors' definition of a basement membrane.

### *Locules*

The locules of the smallest tumors usually were situated beneath the free border of the ovary in the innermost cortical zone away from the hilum of the ovary, but might occur in the medulla adjacent to the cortex. Such primitive locules of uniform or variable size were lined with simple cuboidal or columnar epithelium. Pseudopapillae were common in locules lined by columnar cells. Slightly larger primitive locules of a more irregular outline were lined by simple flat epithelium with gradual transition to tall-columnar cells. On serial section most of these locules were isolated.

The presence of simple flat epithelium in such locules is of some importance. In view of columnar cells elsewhere in the locules, and the irregular outline of the locules, it is unlikely that the existing intralocular pressure is sufficient to cause pressure atrophy. The irregular outline of the locules and the presence of flat epithelium may be interpreted as evidence that such locules were previously under much greater tension.

The cytoplasm of the cuboidal and columnar epithelium stained with mucicarmine in each instance, while that of the flat epithelium stained poorly, if at all. Rarely, islands of small polyhedral cells might be identified in the lining epithelium or in the locular lumina. In no position did the primitive locules approximate the tubules of the rete ovarii, epoophoron, or paroophoron.

In large tumors the locules were more variable in size and distribution and there was no correlation between the size of the locule and the height of the lining epithelium. The cells might be cuboidal, columnar, or flat, with transition to columnar cells. Transition from flat to columnar epithelium was less often observed in larger tumors. The intralocular tension in large locules might result in pressure atrophy of the epithelium. In locules lined with goblet cells, the degree of epithelial compression was evident. In such epithelium the long axis of the goblet was parallel with the basement membrane, rather than vertical. While in the flat epithelium of the primitive locule there were few or no secretory granules



staining with mucicarmine, that of the larger locules contained numerous granules reacting with this stain. In proliferation the epithelium might be tall and prismatic, and an extreme degree of proliferation was present in the epithelium of a cystadenoma removed during pregnancy. Spurs or remnants of septa occurred in the larger locules of older tumors. Multiplication of locules occurred by inclusions of locular epithelium, and in this way satellite locules were formed. In one instance there was a corpus albicans in the wall of a locule. Over the festooned hyalinized mass the epithelium was intimately applied. The cells were flat with transition to tall-columnar epithelium, and numerous secretory granules were demonstrated with mucicarmine. It was impossible to ascertain whether such a locule arose from a corpus albicans cyst, although such cysts have been reported lined by tall-columnar epithelium. The epithelium might be altered by necrosis, degeneration, or inflammation. Within the locules there might be lymphocytes, polymorphonuclear leukocytes, desquamated epithelium, macrophages or cholesterol crystals.

### *Columnar Epithelium*

The epithelium most characteristic of the pseudomucinous cystadenoma was of simple columnar type. Pseudo-stratification usually was the result of a tangential plane of section, but foci of true stratification exist. Even though Novak<sup>7</sup> held that the cells are always simple columnar, Shaw<sup>8</sup> found stratification at some place in 24 per cent of 91 specimens.

The columnar cells measured from 10 to 54  $\mu$  in height and 3 to 8  $\mu$  in width. The size and shape of the individual cell depended on the pressure exerted upon it, the intracellular activity, and the position of the cell in the epithelial layer. The pressure of the intralocular contents of mature tumors might depress the epithelium. In addition, the pressure of adjacent epithelial cells might alter the shape of the cell. While most, if not all, of the cells could be transformed into goblet or chalice cells, their lateral borders frequently remained more or less parallel owing to the pressure of contiguous cells. As will be discussed later, the morphologically typical goblet cells were present in sites where pressure was not great.

The secreting epithelium underwent cyclic alterations with characteristic changes in the cellular configuration. Barzilai<sup>9</sup> recognized separate phases of intracellular activity, and defined secretory, excretory, and resting phases. Her description of the secretory and excretory phases is in keeping with the results of this study, but the description of the resting phase differs somewhat from my findings in this stage. With increasing intracellular secretion the cell might become wider or slightly

pyramidal. On discharge of the secretion the lateral cell borders might become compressed because of the pressure of adjacent cells. There was no evidence that the cells regressed in height after completion of a cycle. Adjacent cells might vary in their phases of activity, and the epithelium of the locule did not necessarily exhibit a uniform cyclic change as is true of the endometrium. Thus there was no apparent correlation between the endometrial cycle and the phase of the cellular activity in this tumor. Comparison was possible in only a few instances since curettage was not usually performed at the time of operation.

The configuration of the cells was altered by fatty degeneration, cloudy swelling, and in proliferation. However, such pleomorphism should not be interpreted as evidence of carcinoma.

### *Terminal Bars*

Terminal bars were demonstrated in Heidenhain's iron-hematoxylin preparations. These were in or on the free border and closed the intercellular clefts on the free surface. When section was horizontal through the free border, the bars appeared as solid bands encircling the cells, but when the section was vertical they were in the form of small nodes or bars at the free border. Maximow and Bloom<sup>10</sup> stated that they are composed of a cementing substance. This may explain the strong cohesiveness of the cells and the fact that they are characteristically more adherent to one another than to the underlying tissues.

### *Striate Border*

A striate border was demonstrated only infrequently on the free margin of the epithelium. It was apparent with hematoxylin and eosin and Heidenhain's iron-hematoxylin staining. The striate border occurred on actively secreting epithelium and consisted of a fine, regular, vertical striation measuring up to  $2.5\ \mu$  in height. Beneath the striate border was a transverse dark line which in some sites could be resolved into small basal granules. Because of the limited number of fresh specimens and the infrequent occurrence of the structure, examination by fresh tissue was not done. Characteristically, however, the striate border does not possess motility. Such a structure is present on intestinal epithelium and has been demonstrated on the epithelium of the placental villus. A comparable structure, *i.e.*, the brush border, is characteristic of the epithelium of the renal tubules. The exact nature of the structure is unknown, although it has been suggested that the striations are the structural expression of water currents entering or leaving the cell. Lucas<sup>11</sup> believed that it is not genetically related to the ciliary apparatus.

### *Ciliated Epithelium*

Careful examination of the fixed preparations in this series revealed no epithelial cilia. This is in accordance with the observations of Shaw<sup>8</sup> and Geist,<sup>12</sup> but Miller<sup>13</sup> and Novak<sup>7</sup> claimed to have demonstrated ciliated epithelium. As previously noted, fresh specimens were not examined for the presence of cilia.

### *Cell Membrane*

The columnar cells possessed a well defined cell membrane. The membrane overlying the free margin of the cell reflected the intracellular activity. Thus, when the amount of secretion was small, the border was flat, but with larger amounts of secretion and increased intracellular tension, it bulged. With discharge of the cell contents, the membrane stained poorly and ruptured. In other cells a small stoma appeared in the cell membrane, which extended through the striate border when that was present. In those cells with a well defined stoma the excretion appeared to occur slowly and a plug of secretion might persist in the stoma. In the tall prismatic epithelium of proliferation, the cell membrane appeared to be wrinkled, but this may be an artifact.

### *Nuclei*

The nuclei varied in size, shape, and intracellular position. In low-columnar epithelium the nucleus might be spherical, averaging  $5.5\ \mu$  in diameter, while in taller columnar epithelium the nuclei were ovoid and averaged  $8\ \mu$  in greatest diameter. Elongated compressed nuclei measured up to  $10\ \mu$  in greatest dimension. In the resting cell the nucleus usually was ovoid and was in the basal aspect of the middle third of the cell. At this stage the nuclear chromatin network was only slightly basophilic and the nucleolus was small, but with increasing secretion the chromatin network became more deeply basophilic and one or, rarely, two prominent nucleoli might be observed. As secretion accumulated in the cell there developed a depression in the superior nuclear pole, and eventually the nucleus might be displaced basally in the cell to be converted into a cup-shaped mass. Other nuclei became flattened and basal in location. Some cells with a poorly defined streamer extending from the inferior nuclear pole to the cell base perhaps were less subject to nuclear displacement. With discharge of the cell secretion, the nuclei became ovoid and returned to the resting position.

The nuclear infolding or grooving in the longitudinal plane was observed especially in tall proliferating cells. Danforth<sup>14</sup> believed that it is more common in lower epithelium. According to Arey,<sup>15</sup> this nuclear

folding was first observed by Varangot, but Danforth was first to identify it in the pseudomucinous cystadenoma. This nuclear change is not specific and occurs in follicular epithelium, endocervical epithelium, and may occur in the cells of Sertoli as well as in other sites.

When studied with Heidenhain's iron-hematoxylin stain the nucleus had a well defined nuclear membrane. A chromatin network was present, sometimes containing small chromatin masses. Although mitotic figures were not often observed, they became numerous when proliferation was active.

### *Cytoplasm*

The cytoplasm usually was diffusely and finely granular, and the granules might be either acidophilic or basophilic. I agree with other investigators that the granules are primarily acidophilic and subsequently become basophilic. As the granules enlarged and were distributed more widely in the cell, they appeared to lie against a clear cytoplasmic background to give the so-called ground glass appearance. The mucicarmine stain was used to study the secretory granules of the epithelium. It should be remembered that while this stain is of some aid in identifying tumors of this type, the cells of the serous cystadenoma may also contain secretory granules reacting with mucicarmine. The granules varied somewhat in their distribution throughout the cycle. In the early stage they were small and supranuclear in location, later filling the theca of the goblet cells and producing a more or less confluent mass of secretion. With increasing secretion the cell cytoplasm became compressed and a poorly defined basophilic zone was apparent about the basal nuclei.

Many of the cells had a demonstrable chromophil substance. With toluidine blue such cells had vertical basal striations, usually situated beneath the nucleus but in some cells extending up about the nucleus. While the origin and function of the chromophil substance is unknown, it is similar to the Nissl substance of nerve cells.

With metachromatic dyes such as cresyl violet and crystal violet, some of the secretory granules exhibited a true metachromatic reaction. These granules were slightly larger and better defined than other granules, and might be observed both within the epithelium and in the locular lumina. In view of the possible entodermal origin of the cystadenoma, some may consider these cells as being similar to those of Paneth. Hertzog,<sup>16</sup> in studying the cells of Paneth, employed de Galantha's amyloid stain,<sup>17</sup> and found the granules to stain similarly to amyloid. While Congo red was employed by Hertzog, its use here was unsatisfactory because of the poor differentiation of the stain. Morphologically, the

granules staining with metachromatic dyes were not unlike those of many types of mucus-producing epithelium. The presence of such secretory granules in goblet cells will be described later.

In addition, when stained with sudan IV, the cytoplasm of the columnar cells contained many minute, finely dispersed fat droplets. With sudan black B there were comparable black granules. This finding was common and is probably not a manifestation of degeneration, because in degeneration the demonstrable fat occurs in larger droplets distributed both throughout the cell and beneath the nucleus.

Only a few specimens were examined for glycogen with Best's carmine technic. A few of the cells contained well defined droplets of glycogen distributed irregularly throughout the cytoplasm.

### *Mitochondria*

Mitochondria were demonstrated with Kull's<sup>18</sup> modification of the Altmann technic. The mitochondria of the columnar epithelium usually were filamentous. The filaments consisted of long, thin, rod-like structures arranged more or less parallel to the long axis of the cell. They might be interwoven and wavy, and extended from the cell base to a site beneath the free border. The filaments adjacent to the nucleus might follow the curvature of the nuclear poles or even encircle the nucleus.

Granular forms of mitochondria also occurred. They are not to be confused with filaments sectioned in a tangential plane. Granular forms usually were below the nucleus but might be above it also.

In the normal cycle, mitochondrial changes were slight or absent, but in degeneration they were noteworthy. In the degenerate cell the mitochondria became fragmented and appeared as short rods and granules without orientation in the cell. In other degenerate cells mitochondria were absent, having undergone chondriolysis. These observations are in keeping with those of Cowdry.<sup>19</sup>

The only conclusions drawn from examination of the mitochondria are as to their type and changes in degeneration. The multiplicity of technical errors in studying such structures makes evaluation difficult.

### *Goblet Cells*

Typical goblet cells occurred in sites where compression was not great. They were numerous in some of the locules and were common over the papillae. Within the theca of the cells there were secretory granules staining with both mucicarmine and the metachromatic dyes. These granules were large, and with Heidenhain's iron-hematoxylin stain were well defined and surrounded by a small, clear halo. A small stoma might

appear in the theca and through this opening the secretion was discharged. Beneath the nucleus there might be a thin projection anchoring the cell to the underlying basement membrane. The goblet cells were not in phase with the adjacent columnar cells and appeared to have their own independent cycle. After discharge of the cell content, the theca collapsed and the cell might become compressed between adjacent columnar cells.

With rapid proliferation of the columnar epithelial layer, these goblet cells might be forced upward and the anchoring streamer broken. Such cells lay high in the cell layer and in serial sections presented no demonstrable connection with the basement membrane, but they still might undergo a cycle in this site. The nuclei of such cells were spherical, deeply basophilic, and lay beneath the free border of the columnar epithelium. In secretion, a small supra-nuclear vacuole was formed. This enlarged and eventually the nucleus was converted into a crescentic mass with overlying theca containing secretion. Rarely there was discharge of the entire cell, including the nucleus and cytoplasm, and thus a discharge of partial holocrine type was present in addition to merocrine secretion. Varying degrees of alteration in polarity might be present, with secretion even occurring beneath the nucleus in some cells. The secretory granules were similar to those of the goblet cells lower in the epithelium.

Between the nuclei of the columnar cells and the basement membrane there frequently were irregular nuclear masses. These averaged  $6\ \mu$  in diameter, were deeply basophilic, and frequently were surrounded by a clear zone of variable width which contained no granules in mucicarmine preparations. Such cells represented leukocytes or perhaps degenerate tumor cells.

Less often in this basal region there were secreting cells with loss of polarity. These were goblet cells which had been displaced downward in the epithelium. Even with rupture of the basement membrane these cells were not detected invading the stroma.

#### *Argentaffin Cells*

Argentaffin cells were first demonstrated in this tumor by Masson.<sup>20</sup> Similar cells in the intestinal tract are spoken of as enterochromaffin cells, basal granular cells, or argentaffin cells. We use the term argentaffin as descriptive of the granules, without implying that the cells are of intestinal origin.

As demonstrated by Masson's technic, the cells were perhaps more common in the parvilocular tumors, but even in that type of cystadenoma

they were not numerous. The cells usually were widely scattered and did not occur side by side. The cell itself might be flattened or flask-shaped, located between adjacent columnar cells or compressed against the basement membrane. The cell nucleus was spherical, averaging  $7\ \mu$  in diameter, and might be situated within or above the granular mass. The surrounding argentaffin granules were small and so closely packed in some cells that the individual granule could not be outlined. The granules varied somewhat in their staining reaction with the silver technic, and both black and brown granules occurred, as noted by Masson<sup>21</sup> in paraffin sections.

The chemical characteristics of the enterochromaffin cells of the intestinal tract have been investigated by Gomori,<sup>22</sup> who believed the granules contain a derivative of resorcinol. According to Macklin and Macklin,<sup>23</sup> similar cells have been identified in the esophagus, stomach, intestine, adrenal, and pancreas.

The origin of the argentaffin cells in this tumor is unexplained. Witschi<sup>24</sup> recently has confirmed the observations of earlier investigators in regard to a migration of the germ cells to the anlage of the gonad. In this study Witschi stated that there is no evidence that other cells accompany the migrating germ cells. The presence of such argentaffin cells in this tumor is not a substantial basis for postulating enteric origin. Further study of these cells is now in progress.

### *Histogenesis*

The literature dealing with the genesis of this tumor is extensive, and since the earlier works are speculative rather than investigative, detailed reference is unjustified. Pflüger's tubules have been implicated by some, but Felix<sup>25</sup> concluded that such tubules do not exist in man. Forbes,<sup>26</sup> in a study of the medullary cords of the human ovary, found only one "medullary tubule" in 55 ovaries examined. He stated further that the medullary cords usually disappear in fetuses of 280 mm. crown-rump length. The tubules of the epoophoron and rete ovarii also have received attention. Goodall<sup>27</sup> suggested the possibility of origin from the germinal epithelium, and also emphasized the association of the tumor with teratomas of the ovary. Bland-Sutton<sup>28</sup> referred to the cystadenomas as embryomas in which gastro-intestinal epithelium predominates. Schiller<sup>29</sup> and most of the contemporary group classify the tumor as a monophyletic teratoma arising from displacement of cells during embryonal life. Novak and Jones<sup>30</sup> suggested that some of the cystadenomas may arise from Brenner tumors. Origin from follicular epithelium is postu-

lated by many writers, but Gardner<sup>31</sup> probably was the first to demonstrate it.

The three smallest cystadenomas in this series, consisting of only a few microscopic locules, were found in ovaries of the polycystic type. The locules were situated in the innermost cortical zone (zona vasculosa) corresponding to the site of earliest follicular maturation, and most of these on serial section were isolated, suggesting but not proving a multicentric origin of the tumor.

One type of primitive locule, common to each of the small tumors and identical with that described by Shaw,<sup>32</sup> is lined by flat epithelium with gradual transition to columnar epithelium. The simple flat epithelium of such locules is not unlike that lining the microcystic follicle in atresia, and contains few or no secretory granules when stained with mucicarmine. Rarely, islands of small polyhedral cells morphologically resembling granulosa occur in the locular epithelium or in the locular lumina. A well defined zone of granulosa as demonstrated by Gardner<sup>31</sup> was not observed in this study. Neither a degenerate ovum nor fragments of zona pellucida were present in any of the locules.

Outside the basement membrane of some locules there was a zone of polyhedral cells with ovoid or spherical nuclei and clear cytoplasm containing numerous droplets of fat. Between the cells of this zone there were numerous capillaries extending to the basement membrane. This zone was morphologically similar to the theca lutein layer of the follicle in regression. Outside other locules there were connective tissue cells containing droplets of fat of variable size, also suggesting thecal origin. Other locules without such features may represent satellite locules. On the basis of these observations in the primitive locules, origin of the tumor in the atretic follicle is strongly suggested.

The current view is that degeneration of the ovum precedes follicular atresia, although Novak<sup>33</sup> once suggested that it depends on discharge of the ovum. Atresia may occur at any stage of follicular development, and Bartelmez,<sup>34</sup> in describing the series of events, stated that the granulosa nearest the atrium of the follicle undergoes degeneration first, while that near the basement membrane degenerates later. Such involution results in the formation of microcystic follicles lined by flat or cuboidal epithelium with or without recognizable granulosa. Such follicles are frequent in the ovary.

In studying ovaries of various age groups, I have found that the liquor folliculi and the zona pellucida of the atretic follicle may stain with mucicarmine. Similarly, with this stain the granulosa cells may show



cytoplasmic secretory granules. This similarity in staining reaction is not unusual since both the zona pellucida and the liquor folliculi are products of the granulosa cells. The germinal epithelium of infant ovaries contains no secretory granules with mucicarmine, but in the adult this layer may stain with mucicarmine.

Serial sections of the smallest tumors revealed no continuity of the germinal epithelium and the locular epithelium. According to Wilson,<sup>35</sup> the human rete ovarii extends only a short distance into the gonad arising from the most deeply lying cells of the early sex gland. Thus there is no reason to suspect origin from the rete tubules, and in no site do the primitive locules approximate these structures. In only one specimen was there a coexisting Brenner tumor, and there is no evidence to suggest origin from this tumor. The association of the cystadenoma with teratomas is of such infrequency as to be inconsequential.

The exact cause of the prosoplastic metaplasia of the epithelium in the production of this tumor is unknown. That the stimulus is hormonal in nature is suggested by the rapid rise in the incidence of the tumor after puberty and its occurrence during the active sexual periods of life.

The epithelium of the cystadenoma is compared to that of the gallbladder, stomach, intestine, and endocervix by other authors. It is similar to other mucus-secreting epithelium in the body, and more nearly resembles intestinal epithelium.

#### SUMMARY AND CONCLUSIONS

The pseudomucinous cystadenoma is a multilocular or unilocular neoplasm lined by epithelium which has the properties of secretion and proliferation. The locules of variable size and number usually are lined by simple columnar epithelium which may have a striate border. In addition, goblet cells, argentaffin cells, and simple flat and cuboidal epithelium may be present.

In the presence of merocrine secretion the epithelium undergoes a cyclic alteration. A secretion of partial holocrine type also occurs in some cells. The secretory granules of the epithelium stain with mucicarmine, and in some instances with metachromatic dyes such as cresyl violet and crystal violet. Within the cellular cytoplasm there may be demonstrable fat, glycogen, and a chromophil substance. The mitochondria are of the filamentous type and may be granular. Mitochondria become fragmented in cellular degeneration.

Evidence is presented to suggest the follicular origin of the cystadenoma. In the development of the tumor either secretion or proliferation may dominate, or the two processes may be in balance.

Enlargement of tumor locules results in a reduction of number and eventually in the formation of a unilocular cystoma.

I wish to express my appreciation to Miss Ethel Lieb for expert technical assistance in this study.

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## DESCRIPTION OF PLATES

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### PLATE 105

FIG. 1. Lining epithelium of a multilocular cystadenoma, showing variations in phase of merocrine secretion. Some nuclei are compressed between adjacent cells; others have a supranuclear vacuole. There is longitudinal grooving of several nuclei. Of note are the basal nuclear masses. Terminal bars close the intercellular clefts in the free border. Heidenhain's iron hematoxylin stain.  $\times 1065$ .

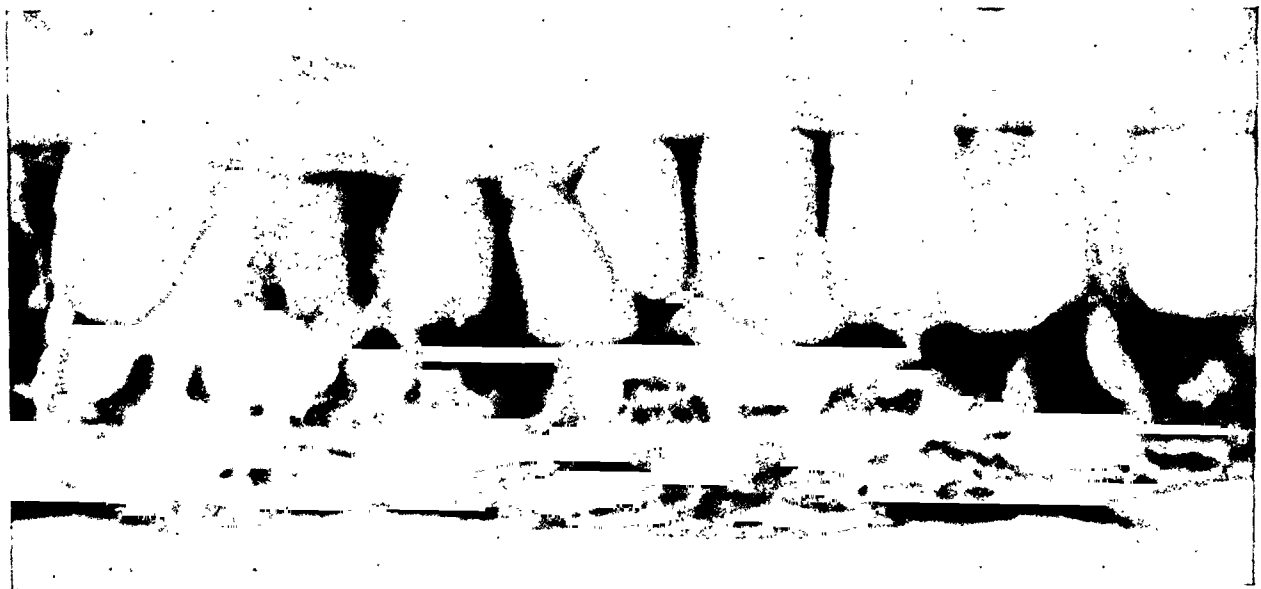
FIG. 2. Lining epithelium of a multilocular cystadenoma, made up of goblet cells, several of which are in merocrine excretion. A striate border is present. Heidenhain's iron-hematoxylin stain.  $\times 1065$ .

FIG. 3. Lining epithelium of a parvilocular cystadenoma, showing two argentaffin cells. Of note is the nuclear infolding. Three goblet cells lie high in the epithelial layer. Masson's stain.  $\times 1120$ .

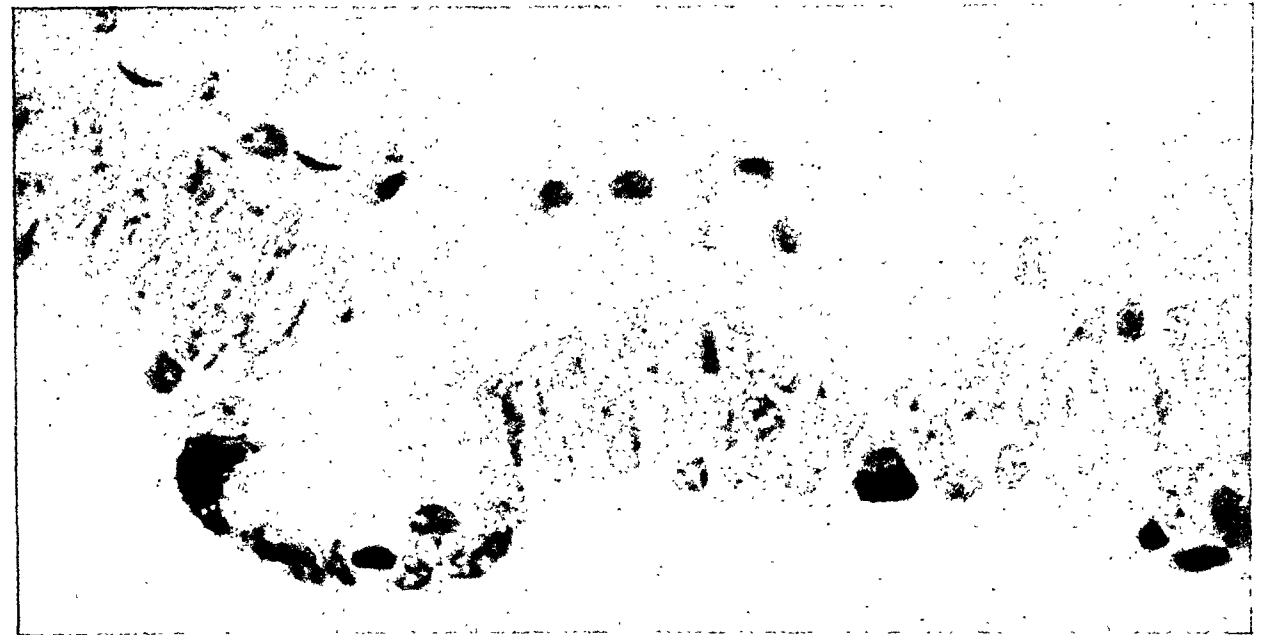
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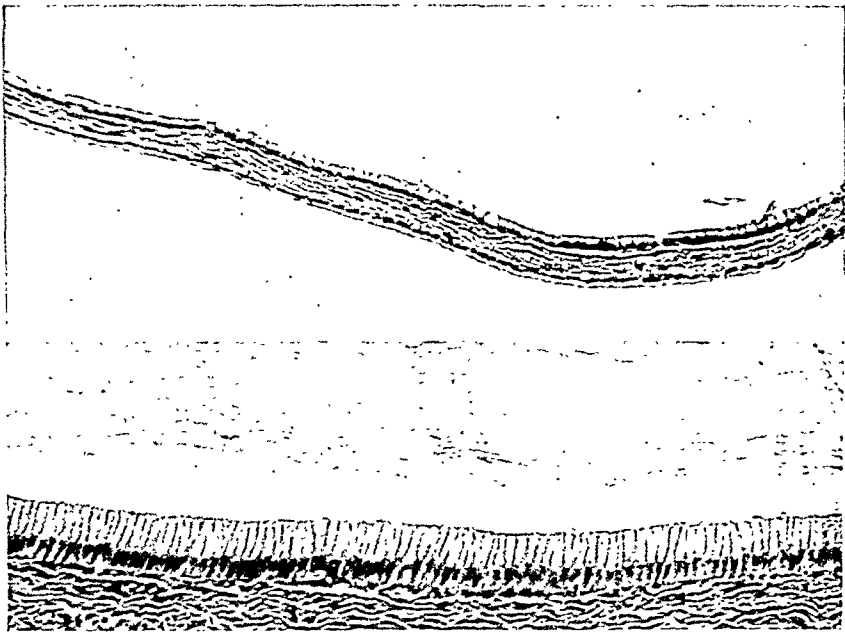
Pseudomucinous Cystadenoma

PLATE 106

FIG. 4. The flat epithelium lining the septum, and the columnar epithelium beneath are from the same locule. There is variation in height, even in the same locule. Hematoxylin and eosin stain.  $\times 117$ .

FIG. 5. Cystadenoma of papilliferous type. Of note are the goblet cells over the papillae. Hematoxylin and eosin stain.  $\times 770$ .

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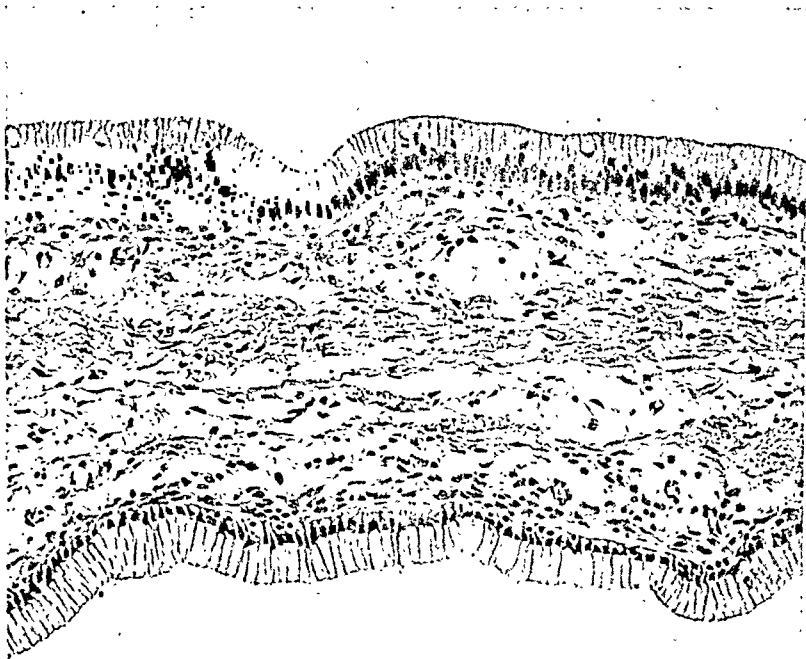
Pseudomucinous Cystadenoma

PLATE 107

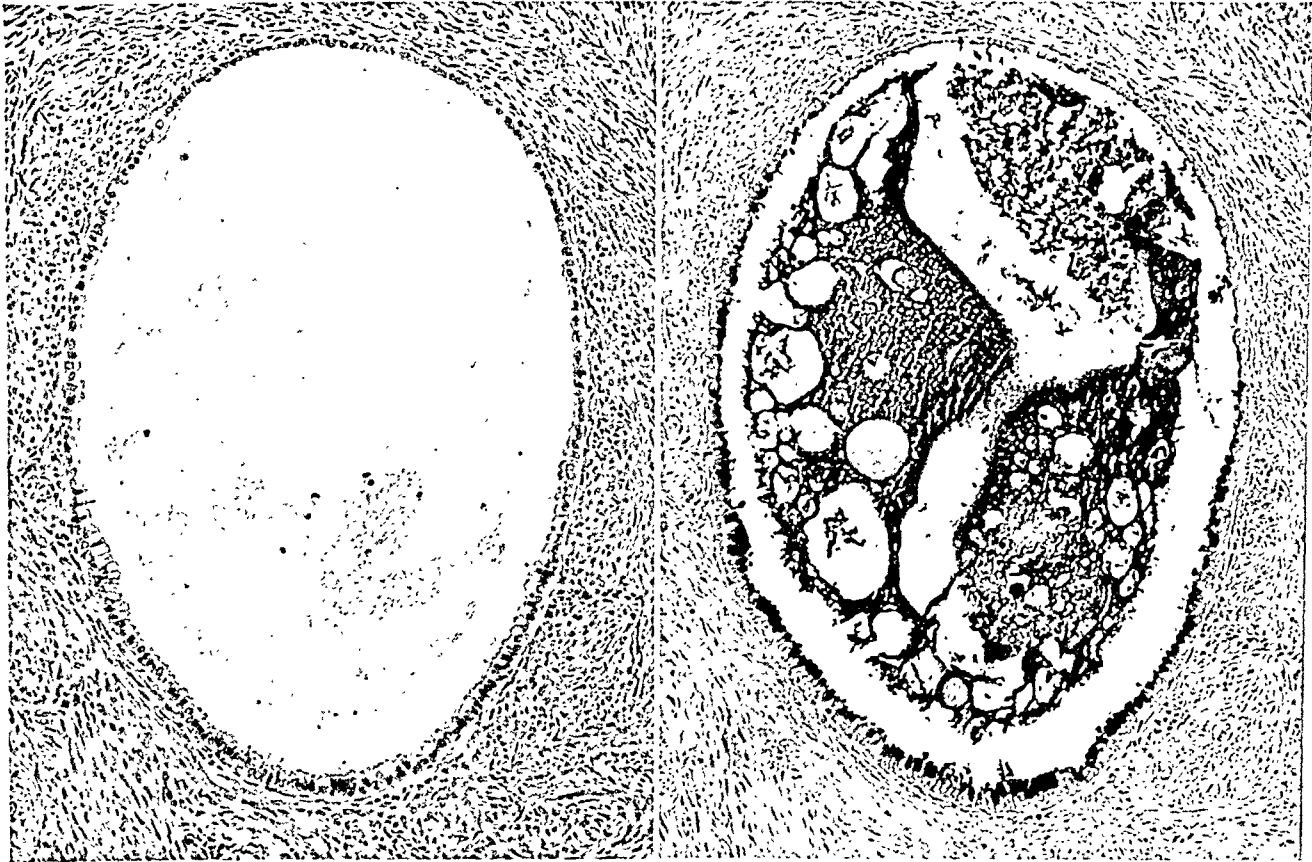
FIG. 6. Epithelium lining septum of multilocular cystadenoma. Goblet cells lie beneath the free border of the upper epithelial layer. The lower epithelial layer shows the so-called ground glass appearance of the cytoplasm. Hematoxylin and eosin stain.  $\times 177$ .

FIG. 7. Primitive microscopic locule from a small cystadenoma. Of note is the gradual transition from flat to columnar epithelium. Hematoxylin and eosin stain.  $\times 150$ .

FIG. 8. Same primitive locule as seen in Figure 7. The columnar epithelium contains abundant secretion while the flat and cuboidal cells contain little or no secretion. Mucicarmin stain.  $\times 150$ .



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Pseudomucinous Cystadenoma





## EXPERIMENTAL INFARCTION OF BONE AND MARROW\*

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The long bones of mammals receive their blood supply from three sources. These are: (1) multiple small periosteal vessels penetrating and supplying the outer cortex; (2) epiphyseal vessels entering the ends of bones through ligamentous and capsular attachments; and (3) one (or more) nutrient arteries entering the shaft through nutrient foramina and supplying the diaphyseal marrow and inner cortex. There is considerable evidence that these vessels anastomose freely, particularly in the adult following disappearance of the epiphyseal cartilage plate, and it is for this reason that the possibility of bone or marrow infarction is often denied. Haslhofer,<sup>1</sup> writing on the circulation in bone and marrow in the Henke-Lubarsch Handbuch, stated that because of the richness of these anastomoses, disruption of even the largest source, *i.e.*, the nutrient artery, produces no sequelae, even in youth.

Clinicians, on the other hand, at times invoke infarction to explain otherwise cryptic bone lesions. Axhausen and Bergmann,<sup>2</sup> writing in the same volume with Haslhofer, presented clinical instances of aseptic bone necrosis which they ascribed to interruption of local blood supply. Phemister and his associates<sup>3,4</sup> have published radiographic and pathologic descriptions of lesions which they considered the result of marrow infarction. Some of the lesions occurred in patients who had previously had caisson disease.

Because the production of bone and marrow infarcts in animals has generally been considered impossible by conventional means, earlier investigators resorted to extensive stripping procedures or to the production of multiple small emboli designed to occlude large numbers of capillaries. Brunschwig,<sup>5</sup> attempting to produce infarction of the marrow of the femur in dogs, stripped the entire periosteum and simultaneously cut the nutrient artery. Despite this extensive trauma, he found no evidence of infarction in 15 adult dogs. In one young animal followed for 60 days, he described fibrosis and cyst formation within the marrow cavity. Among injection experiments may be mentioned those of Wollenberg,<sup>6</sup> who injected talc into the femoral artery of dogs and observed areas of necrosis in metaphyses and epiphyses. Bergmann,<sup>7</sup> on the other hand, following the injection of particles of silver suspended in gum arabic, found no changes in the epiphyses but widespread necrosis of

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cortical bone. Kistler,<sup>8-10</sup> having failed to observe infarcts following simple ligation of the nutrient artery of the femur in rabbits, finally resorted to injecting suspensions of charcoal in acacia and masses of agglutinated bacteria. He injected these directly into the nutrient artery under pressures which were not measured but which were admittedly high. By these means he was able to produce areas of necrosis in the center of the metaphyses.

Huggins and Wiege<sup>11</sup> were the first to report changes following disruption of the nutrient vessels alone. In both mature and immature rabbits, ligation of the nutrient vessels to the femur was followed in all instances by infarction of marrow. Although in a few cases there was some periosteal and endosteal reaction about the operative site, they found no evidence of infarction of bone.

In view of the non-physiologic means usually employed previously and because of the absence of uniformity in the results obtained, it was decided to attempt once more, under better controlled conditions, the experimental production of bone and marrow infarcts.

### METHODS

Large, but skeletally immature, rabbits weighing between 2.3 and 3.3 kg. were subjected to transection of the nutrient artery of the femur. Immature animals were used throughout because, as shown by Drinker, Drinker, and Lund,<sup>12</sup> so long as the epiphyseal cartilage plate remains, anastomoses between diaphyseal and epiphyseal vessels are at a minimum.

The animals were anesthetized with intraperitoneal injections of sodium pentobarbital, supplemented when necessary with ether inhalation. Using sterile technic, the nutrient artery was exposed and cut as close to the bone as possible. As one or two small veins usually accompany the artery, they were inevitably destroyed as well. A description of the gross anatomy of the blood supply to this bone may be found in Kistler's first paper.<sup>8</sup> The distribution of arteries within the bone and their relative size are illustrated in Figure 8 of this paper. Bleeding was minimal and as a rule no ligatures were necessary. The wound was closed with cotton sutures. In one case, because of faulty technic, the wound became infected; this animal was discarded. As our primary purpose was to follow the cytologic sequence of events after infarction of the bone and marrow, most animals were operated upon bilaterally.

The animals were sacrificed with chloroform or air embolism at intervals of from 24 hours to 6 months. The femurs were cleaned and roent-

genograms were taken. They were then sawed transversely through the shaft and longitudinally through the epiphyses into fragments not over 2 cm. in length. These were fixed in Zenker's fluid or formalin, decalcified in nitric acid, embedded in celloidin, sectioned and stained with hematoxylin and eosin and Mallory's aniline blue. A total of 25 rabbits comprised the basis for this report.

## RESULTS

In practically every femur of which the nutrient artery had been severed, microscopic examination of diaphyseal marrow revealed evidence of infarction. Lesions were most common at the junction of the middle and lower thirds of the shaft, although in the majority of cases there was patchy infarction throughout most of the diaphysis. In several instances the lesions extended into the metaphyses, but epiphyseal marrow was never involved. The infarcted areas frequently abutted upon and involved portions of cortical bone.

The sequence of events within the infarcted marrow was as follows: 24 hours after injury, swelling and diminished staining capacity of hematopoietic cells as well as hemorrhage of slight to moderate degrees were seen. At 48 hours the infarcted areas were outlined by the appearance at the periphery of neutrophils and mononuclear cells containing cellular debris and hemosiderin. By the fourth day lack of uniformity of fat droplets gave evidence of necrosis and rupture of fat cells. At 1 week (Fig. 1), although the larger lesions still showed complete necrosis at their centers, there was considerable cellularity at the periphery. Large numbers of eosinophils made their appearance. The larger fat droplets were being actively phagocytosed by multinucleated giant cells. There was some increase in vascularity in this zone, but it was not prominent.

At 2 weeks the center of the infarct consisted of a very loose fibrillar and granular matrix from which cellular debris had largely disappeared and in which most of the fat droplets had been phagocytosed (Fig. 2). About the periphery there was a well defined zone 1 to 2 mm. wide resembling a granuloma (Fig. 3). Large mononuclear and giant cells predominated, but there were also numerous lymphocytes, eosinophils, and occasional focal accumulations of neutrophils. Connective tissue stains showed no fibrosis. An interesting feature at this stage was the presence of shrunken and pyknotic megakaryocytes completely engulfed by giant cells, a phenomenon which indicates that these cells are unusually resistant to lytic enzymes.

By 3 weeks the lesions again became less well defined by reason of the disappearance of many of the inflammatory cells. During the third and fourth weeks the impression was gained that fat-laden macrophages gradually became indistinguishable from normal fat cells. They appeared to lose their stainable cytoplasm and to assume more uniformity in size. By the eighth week healing had advanced to the point that only careful scrutiny would permit recognition of the lesion. The fat-containing cells were still somewhat more variable in size, tending to be larger than those in the surrounding uninfarcted areas. Interspersed among them were macrophages containing a faintly yellow, globular to crystalline material (Figs. 6 and 7). This substance, often present in considerable quantities, was insoluble in most of the usual fat solvents. It was not doubly refractile, even in frozen sections of formalin-fixed material, and was not acid-fast. It stained blue with Nile blue sulfate, and, although unidentified, is probably a lipid.

By 6 months, the previously infarcted areas could be identified with certainty only by the presence in them of macrophages containing this material. These cells were interspersed among what appeared to be normal fat cells. Such areas were without hematopoietic activity. Their most noteworthy characteristic, however, was the complete absence of fibrous scarring.

One animal developed diarrhea, lost weight rapidly, and died 12 days following surgery. Because of starvation the normal marrow was depleted of fat, its place being occupied by engorged sinusoids. The areas of infarction were readily recognizable against this background by the continued presence in them of fat. Figure 4 illustrates a section of femur from this animal.

In most cases there was infarction of bone as well as marrow. It was very common to see small areas of necrotic bone along the inner margin of the cortex of the shaft. Such areas occurred only where a marrow infarct abutted on cortical bone. (Infarcted bone could be recognized as early as 48 hours following loss of blood supply because the bone cell nuclei stained less deeply. Within 1 week the area appeared completely acellular.) When the marrow infarcts extended to involve distal or proximal metaphyses, there was necrosis of bony trabeculae within the zone of provisional ossification. In one case, 2 weeks following injury, there was evidence of a disturbance in longitudinal growth sequences.

The infarcted bone elicited no specific reaction. When the area involved was small, as was usually the case, it was gradually resorbed and replaced. As all bones were still growing at the time of infarction,

remodeling sequences frequently gave the appearance of necrotic bone incorporated within the mid-zone of cortex. If, on the other hand, a small necrotic area, because of its location, was destined to disappear completely during remodeling, it appeared more resistant to resorption than adjacent viable bone. When the area of infarcted bone was large, as happened less frequently, new bone was rapidly laid down, by periosteum and/or endosteum, in such a manner as to buttress the necrotic, and presumably weaker, area (Figs. 5 and 6).

Because of the small size of most of the bone infarcts, roentgenographic interpretation usually was equivocal. In Figure 9, however, may be seen a definite zone of reaction about an area of necrotic cortical bone.

#### SUMMARY AND CONCLUSIONS

Cutting the nutrient artery to the femur of the growing rabbit almost invariably resulted in infarction. This confirms the work of Huggins and Wiege<sup>11</sup> but is contrary to the experience of others. Infarction was most extensive in the diaphyseal marrow, extending in some cases well into the metaphyses. Epiphyses were never involved. In most instances there were small areas of bone infarction as well. The latter usually were limited to the inner zone of the diaphyseal cortex. Based on a small number of cases in which infarction extended into the metaphyses, it appears that any interference with longitudinal growth sequences was temporary.

The tissue sequences in both marrow and bone up to 6 months following injury are reported. In the case of marrow infarcts, the most striking feature was the complete absence of a fibrous cicatrix. Fat droplets released from necrotic fat cells were taken up by large phagocytic cells, which in time appeared indistinguishable from normal fat cells. The area of marrow infarction eventually was recognizable only by the absence in it of hematopoiesis and by the prolonged presence of numerous macrophages containing an unidentified yellow material, presumably a lipid.

Small areas of infarcted bone excited no reaction other than their very slow replacement by viable bone, a process comparable to "creeping substitution" in bone grafts. Depending on the direction of remodeling processes, they either became incorporated within viable bone or became resorbed. Resorption appeared somewhat slower than in the case of viable bone. When the area of infarction was larger, there was a rapid overgrowth of new bone, designed apparently to buttress the structurally weaker area of necrosis.

Except for the work of Huggins and Wiege,<sup>11</sup> whose interpretation of marrow sequences is somewhat comparable to ours, it is difficult to reconcile our results with those of other authors. Perhaps Kistler,<sup>8-10</sup> who has performed the most extensive work in this field and who failed to detect any changes following ligation of the nutrient artery alone, relied too much on roentgenographic evidence of damage. Brunschwig,<sup>5</sup> who did produce infarction of both marrow and inner cortex (by completely stripping the periosteum as well as cutting the nutrient artery of the femur in young dogs), reported fibrosis and cyst formation in the marrow. This is completely inconsistent with our results. Such results occurred in only one case, and unfortunately he published no photographs.

The human lesions described by Phemister and his associates<sup>3,4</sup> and ascribed by him to marrow infarction, also differ significantly from our experimental lesions. Roentgenographic and pathologic examination in his cases revealed in the femoral marrow widespread areas of liquefaction necrosis surrounded by a zone of calcification. This lack of similarity to our experimental lesions might be explained by differences in bone age at the time of injury, by differences in the duration of the lesions, or, more likely, by the much larger area of infarction permissible in human marrow. Size of infarcts has much to do with the manner in which they heal, or fail to heal.

Comparisons with osteochondritis dissecans and Perthe's disease, syndromes which have frequently been ascribed to infarction, are not pertinent, since in our rabbits there was presumably no interference with the blood supply to the epiphyses. Other examples of aseptic bone necrosis in the human may or may not be comparable.

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[ *Illustrations follow* ]

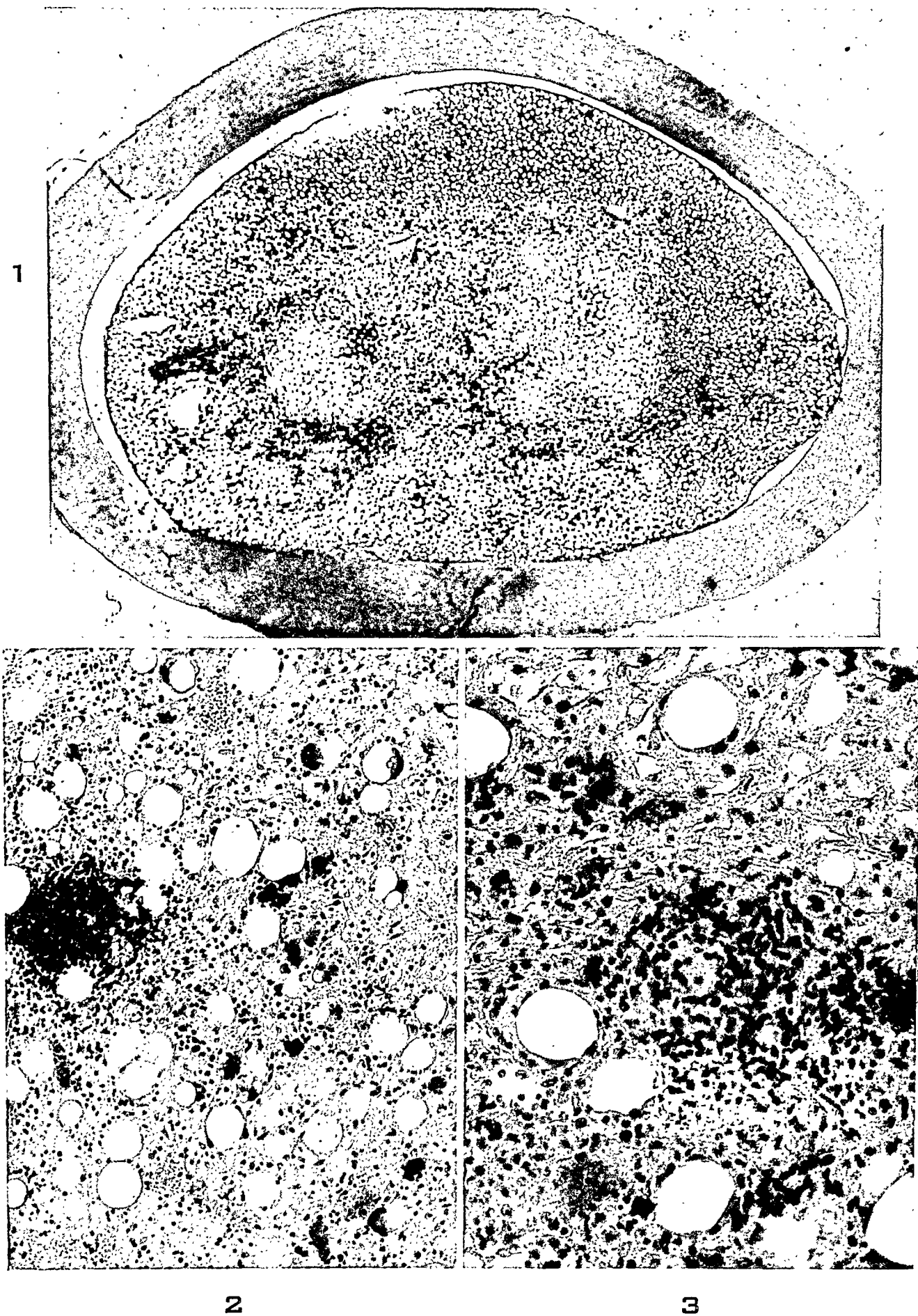


## DESCRIPTION OF PLATES

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### PLATE 108

- FIG. 1. Rabbit 191. Section through the junction of the middle and lower thirds of the shaft of a femur. The nutrient artery was cut 1 week prior to sacrifice. A large marrow infarct is well outlined by a zone of cellular reaction.  $\times 29$ .
- FIG. 2. Rabbit 190. Section of a marrow infarct of 2 weeks. Giant cells may be seen about fat droplets and *engulfing pyknotic megakaryocytes*.  $\times 166$ .
- FIG. 3. Rabbit 189. Section through a zone of reaction about a marrow infarct of 2 weeks. There is a collection of large mononuclear cells. The larger fat droplets are surrounded by a layer of the same cells.  $\times 332$ .



Bragdon, Foster, and Sosman

Experimental Infarction of Bone

PLATE 109

FIG. 4. Rabbit 180. Section of a marrow infarct of 12 days. This animal developed diarrhea, lost much weight, and died. In the uninfarcted marrow the fat has been mobilized and replaced by engorged sinusoids. Within the infarcted area fat droplets have been retained. Small areas of necrotic cortex may be seen along the margin of the marrow infarct.  $\times 32$ .

FIG. 5. Rabbit 183. Section of marrow and cortical infarcts of 4 weeks. The zone of reaction about the marrow infarct has largely disappeared. An area of necrotic cortex has been buttressed by local overgrowth of new endosteal bone, forming an "internal splint."  $\times 32$ .

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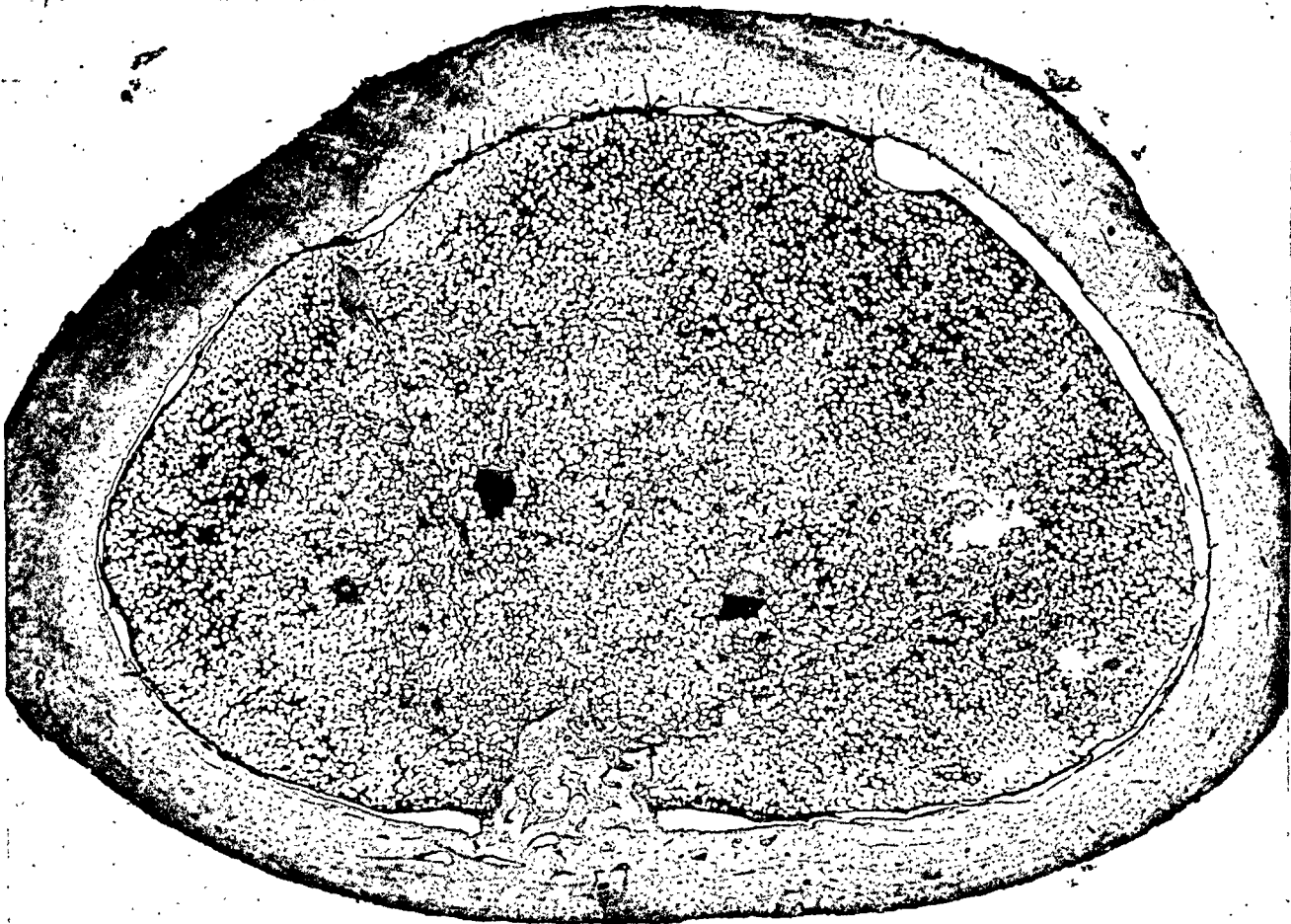
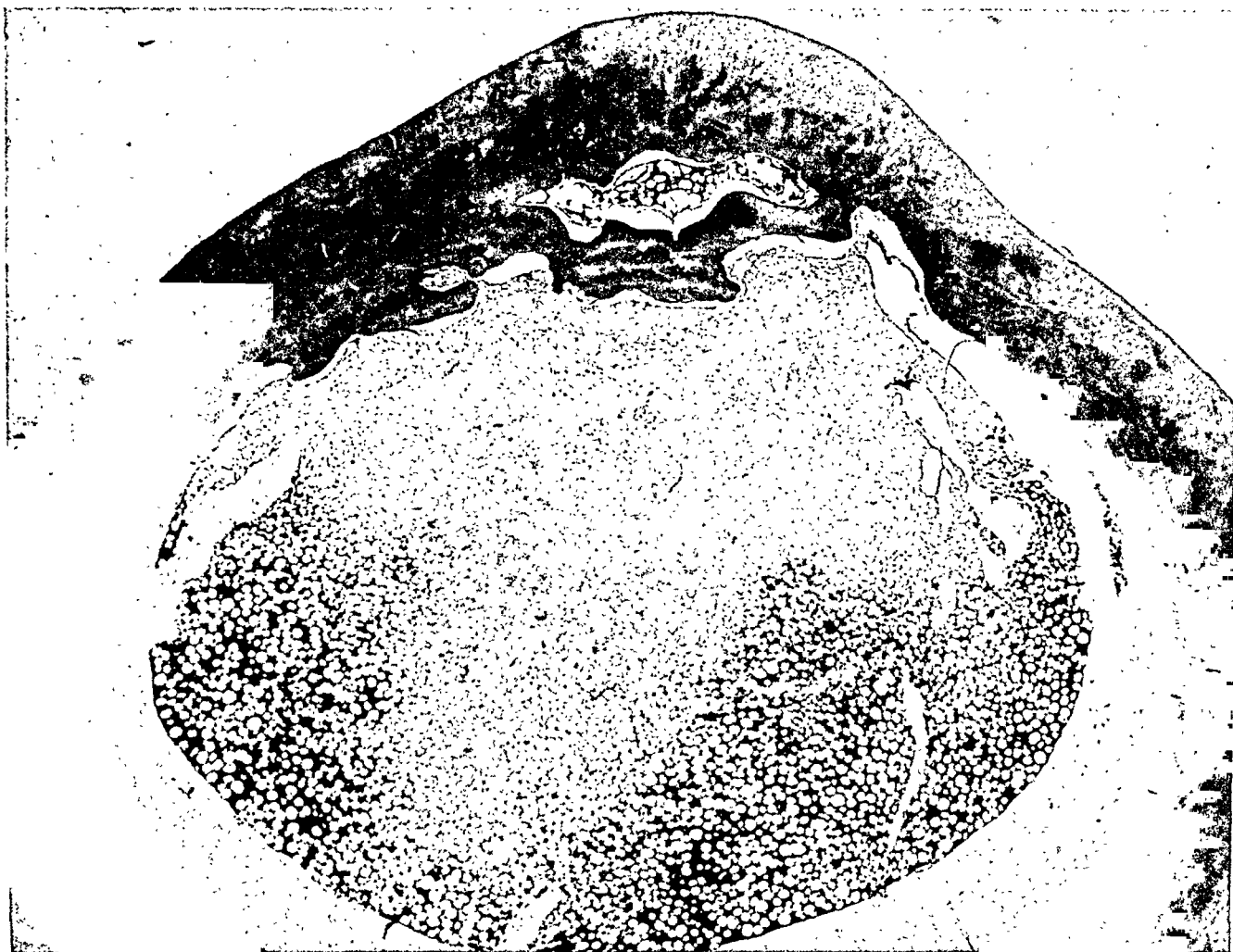


PLATE 110

FIG. 6. Rabbit 157. Section of marrow and bone infarcts of 4 months. The marrow infarct is now recognizable only by the presence of macrophages filled with an unidentified yellow material and by the absence of hematopoiesis. There is no fibrous cicatrix. The infarcted area of cortex has been buttressed by an overgrowth of new bone from the periosteal surface.  $\times 31$ .

FIG. 7. Rabbit 157. Same section as shown in Figure 6. Portions of necrotic cortex still remain after 4 months. There is no evidence of active bone formation or destruction. A small field of infarcted marrow in the lower right corner shows macrophages containing an unidentified opaque yellow material.  $\times 135$ .

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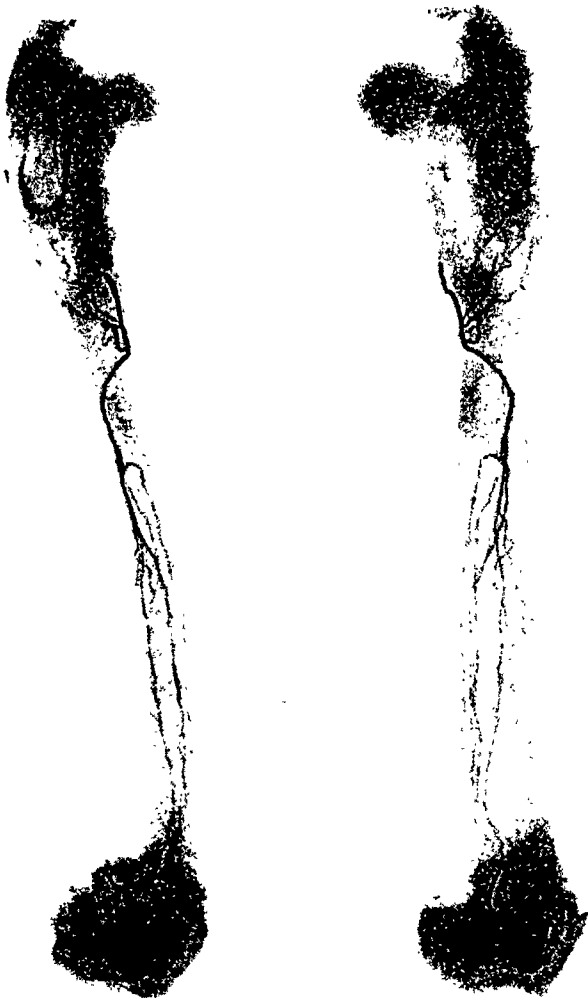


PLATE III

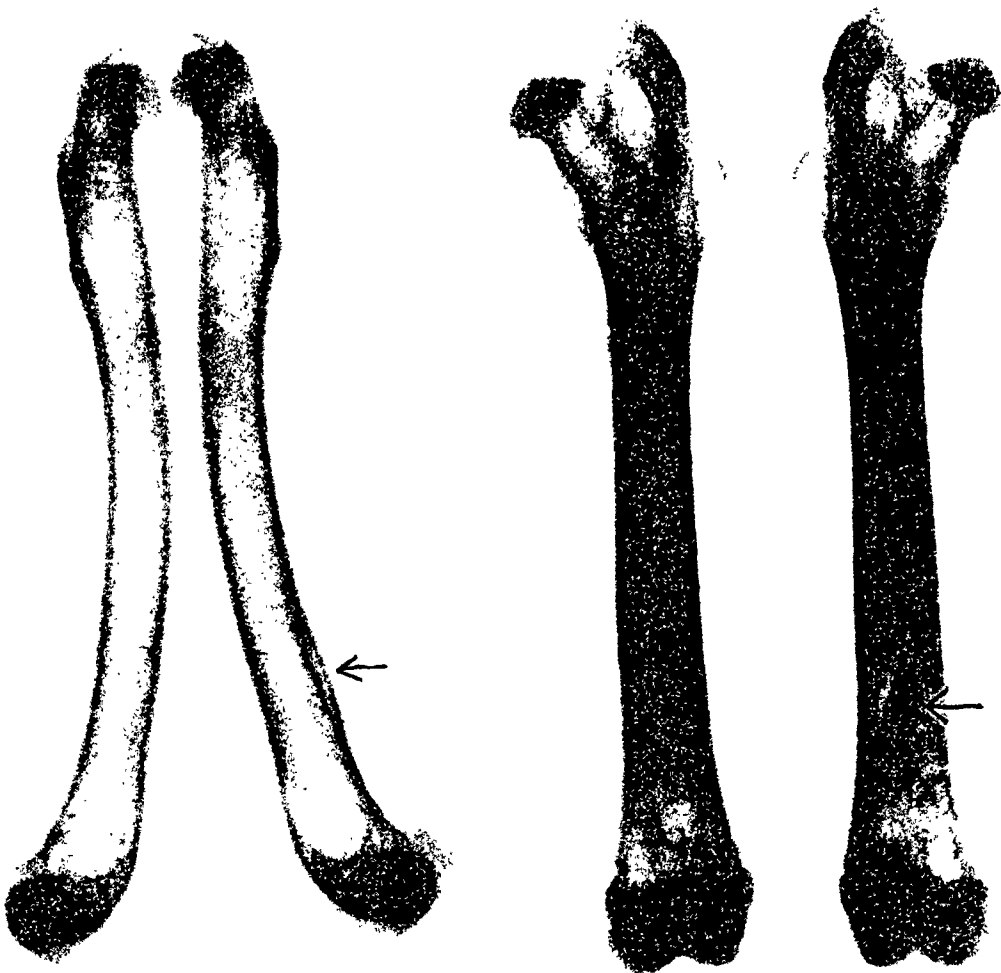
FIG. 8. Anteroposterior and lateral roentgenograms of the femur of a normal, immature rabbit following injection, via the abdominal aorta, of a radio-opaque fluid. The bone has been cleaned and partially decalcified. The small periosteal vessels did not fill, but several epiphyseal arteries, as well as the large nutrient artery and its branches, are delineated.

FIG. 9. Rabbit 157. Anteroposterior and lateral roentgenograms of both femurs 4 months after cutting the nutrient arteries and showing on the right an area of necrotic cortex buttressed by overgrowth of subperiosteal bone. (For comparison with Figures 6 and 7.)

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THE TOPOGRAPHIC PATHOLOGY OF THE CEREBRAL LESIONS  
OF ACUTE POLIOMYELITIS IN MAN  
I. THE MEDULLA OBLONGATA\*

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Our purpose in undertaking this study was to determine in detail the topography of the cerebral lesions of acute poliomyelitis based upon the systematic examination of a sufficiently large number of specimens to furnish a true average picture of the pattern of the disease, and to place the peculiarities of individual cases in proper perspective. It is recognized that this plan is not highly original. During the past several decades there have been numerous reports on the pathology of poliomyelitis. Many of these are based on one or a few cases, examined with varying thoroughness. The relatively few more detailed studies fall generally into two groups. One presents rather comprehensive conclusions based upon examination of random sections from a considerable number of cases. In the other the authors have subjected one or two specimens to a fairly intensive examination, generally directed toward confirming some one theory of pathogenesis. We have encountered only two reports in which a substantial number of brains were examined systematically and the results reported in adequate detail. One is the careful and elaborate investigation of Horányi-Hechst<sup>1</sup> based upon 24 complete central nervous system specimens from the 1931-1933 outbreaks of poliomyelitis in Budapest. The other is a description by Swan<sup>2</sup> of 6 brains from South Australia in 1937. Although only one lateral half of each brain was sectioned, the results are presented in considerable detail. Where possible we shall compare the findings of these two authors with our own in the discussion to follow.

For reasons of convenience we have arbitrarily divided this study into three sections: lesions in the medulla oblongata, described in this paper; lesions in the pons and cerebellum, and lesions in the midbrain, basal ganglia, and cerebrum to be discussed in two subsequent publications.

MATERIAL

Our results are based upon the examination of 42 specimens of brain. Of these, 6 were collected in Chicago in 1936, and 10 from the same city in 1937. From Detroit 8 were obtained in 1939 and 7 in 1941. The re-

\* Investigation supported in part by a grant from the National Foundation for Infantile Paralysis, Inc.

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maining 11 were obtained singly or in small groups from random localities in the eastern part of the United States during 1941.

Clinical histories of varying degrees of completeness were available for 35 of the 42 specimens examined. For the remaining 7 specimens no clinical information of any sort was obtained. Twenty-four of the 35 patients were known to be males and 6 females. A reason for the large preponderance of males was not apparent. Practically two-thirds of the patients presented poliomyelitis of a pure bulbar type and died without the appearance of paralysis of the limbs. The remaining one-third were considered to be of mixed bulbar and spinal type. Most of these were admitted with bulbar symptoms, and toward the end developed some degree of paralysis of the limbs, usually of the arms. In only a few was there initially severe limb paralysis followed by bulbar collapse. The patients ranged in age from 1 to 33 years, but almost all were under 20. Of the 35 patients with known histories, 13 were in the first decade of life, 20 were in the second decade, and only 2 in the third decade. The duration of the disease, based upon the stated dates of onset of symptoms and of death, averaged 5 days for the bulbar type and 8 days for the mixed bulbar and spinal type. The extremes are presented by one patient with poliomyelitis of the fulminating bulbar type who died apparently within 24 hours after initial symptoms, and another with spinal, followed by bulbar, involvement who succumbed 20 days after the onset of paralysis of the limbs.

#### TECHNICAL METHODS

All specimens of brain were fixed in 10 per cent formalin for several weeks or more, preferably and usually without previous sectioning, since the anatomic pattern rather than the cytology of poliomyelitis was the objective of the study. After sufficient hardening, the brain stem and cerebellum were separated from the hemispheres by a transverse incision through the cerebral peduncles just rostral to the anterior colliculi. The hemispheres were separated from one another by a longitudinal incision through the midline of the corpus callosum and the infundibulum. Then both hemispheres and the brain stem and cerebellum were sliced transversely at right angles to their axis at a series of predetermined levels. Blocks for embedding were prepared from each slice and marked with a code number identifying level and side of each. In all, 97 large blocks were so prepared from as nearly identical areas as possible from each specimen of brain. The location of these standard blocks will be indicated in the description of the lesions found in each brain area. Supplemental blocks occasionally were added to the standard series in order to display certain structures more satisfactorily.

After trimming and marking the blocks they were hardened for 3 days in 3 per cent aqueous potassium bichromate solution, washed overnight in running water, and embedded in paraffin. Sections 8  $\mu$  thick were stained routinely by the azure-eosin method of Lillie<sup>3</sup> which gave very satisfactory survey preparations. These were supplemented occasionally by special stains such as those for myelin and reticulum. Each slide was examined completely by means of a mechanical stage, and the types and locations of lesions were recorded.

For the examination of the medulla, sections were prepared routinely from four blocks cut respectively through the decussation of the pyramids, through the medulla at the level of the closure of the fourth ventricle, through the mid-portion of the inferior olives, and through the anterior end of the medulla at the emergence of the eighth nerve roots.

### OBSERVATIONS

In the following description the various nuclei and cell groups are arranged in accordance with the generally accepted embryologic theory of nerve components. Those belonging to the somatic motor component are described first, followed in order by those of the branchial and visceral motor components, and the visceral and somatic sensory components. The medial and spinal vestibular nuclei, representing the special sensory component in the medulla, will be discussed subsequently with related structures in the pons, although for completeness lesions in the part of these nuclei appearing in our most rostral block are indicated in Text-Figure 4.

In describing the cellular changes in this series of specimens we have recognized and recorded lesions of three sorts. The first and most widespread is an infiltration of small lymphoid cells and some mononuclear cells in the Virchow-Robin spaces, forming perivascular collars of varying prominence. These are represented by small circles in the accompanying anatomic diagrams (Text-Figures 1 to 4). The second is a proliferation and accumulation of microglial cells in the ground substance, sometimes diffuse but most commonly in nodular aggregations of varying compactness. These are represented by groups of dots on the diagrams. Some of the smaller dense foci undoubtedly mark the site of destroyed nerve cells, but many of the larger, more diffuse groups appear to be a response to the injurious action of the virus on the ground substance independent of nerve cell destruction. The third lesion, necrosis of nerve cells, is symbolized by small crosses on the diagrams. These necrotic cells appeared as somewhat shrunken oxyphilic masses often lying in a vacuole. Occasionally such dead cells were undergoing neuron-

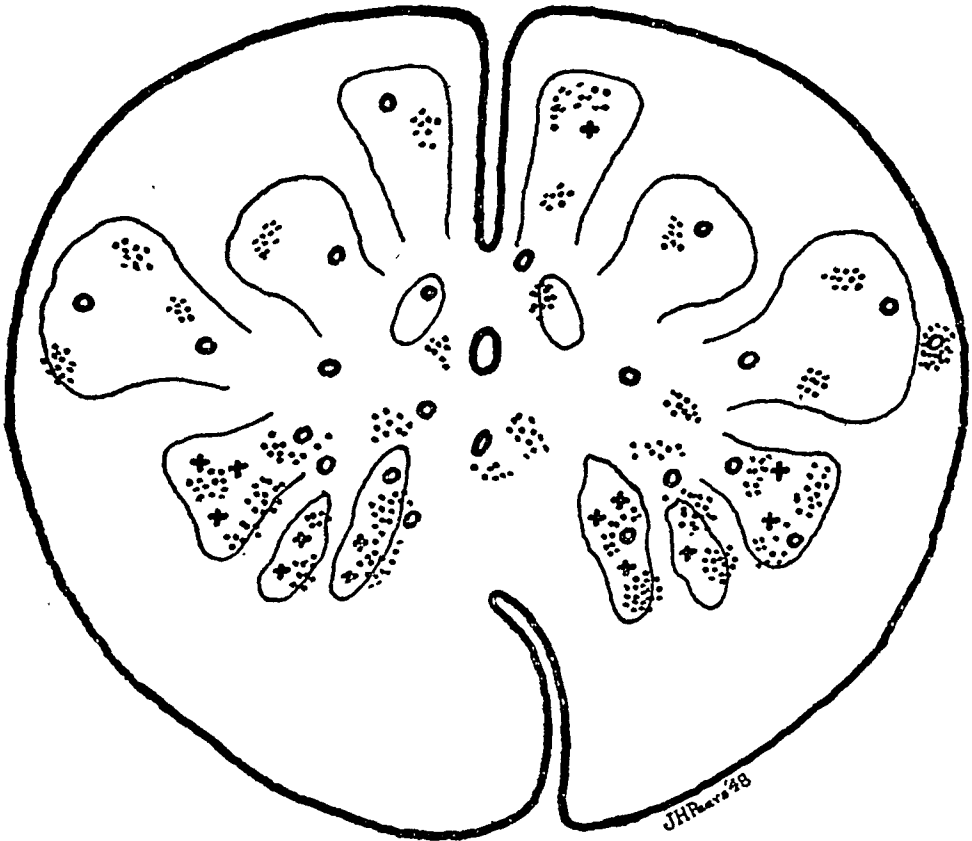
ophagia by phagocytic cells which in most specimens were mononuclear cells of microglial origin. In only a few specimens were polymorphonuclear leukocytes clearly identifiable among the reacting cells. In general neuronophagia was a much less prominent feature in the medulla than in the spinal cord, and many dead cells lay in a spongy ground substance among diffusely infiltrating microglia without exerting any evident chemotactic activity. Lesser degenerative changes such as partial chromatolysis and nuclear eccentricity were not recorded, partly because of the varying state of preservation of our specimens, and partly because of the difficulty of assessing the significance of such minor morphologic variations in nerve cells other than those of large somatic motor type. Only a rough estimate of the amount of cell loss was attempted in large-celled nuclei having a fairly regular and constant structure.

The *nucleus supraspinalis*, the most median of the groups forming the anterior horn column of large somatic motor cells, contains the cells of origin of the first cervical nerve supplying the small deep muscles of the upper part of the neck. Moderately severe lesions were present in this cell group in every specimen examined, irrespective of the duration of the disease or the outbreak from which the specimen was obtained (Text-Fig. 1). Perivascular round-celled infiltration and focal and diffuse interstitial infiltration were moderately abundant. It was estimated roughly that from one-half to two-thirds of the nerve cells had disappeared. In the great majority of the specimens a few necrotic cell bodies were still to be seen, occasionally undergoing active neuronophagia, chiefly by phagocytic microglia. In a single specimen of approximately 11 days' duration both cells and ground substance of the entire horn on both sides had been destroyed. The resulting small cavities were crowded with phagocytic microglia filled with lipoid debris, forming foam cell abscesses.

The nucleus of the *spinal accessory* nerve forms a small discontinuous strand of nerve cells of motor type, lateral to the supraspinal nucleus and extending upward from about the sixth cervical segment for a short distance into the lowest level of the medulla. Its cells supply the motor innervation for the sternomastoid muscle and a large part of the trapezius muscle. By some, the nucleus is considered to form the most caudal group of the branchial motor column. Lesions in this nucleus were moderately severe in all specimens and corresponded closely to those in the immediately adjacent nucleus supraspinalis (Text-Fig. 1). Perivascular and interstitial infiltration was prominent. Occasional necrotic cell bodies were seen in almost all specimens but the degree of cell loss

could not be estimated fairly because of the small and variable size of this nucleus in the medulla.

The *hypoglossal nucleus* is a well defined group of typical large nerve cells of motor type, located just beneath the floor of the fourth ventricle. These cells furnish the motor innervation of the muscles of the tongue. Damage was of about equal degree at the level of the ventricular closure

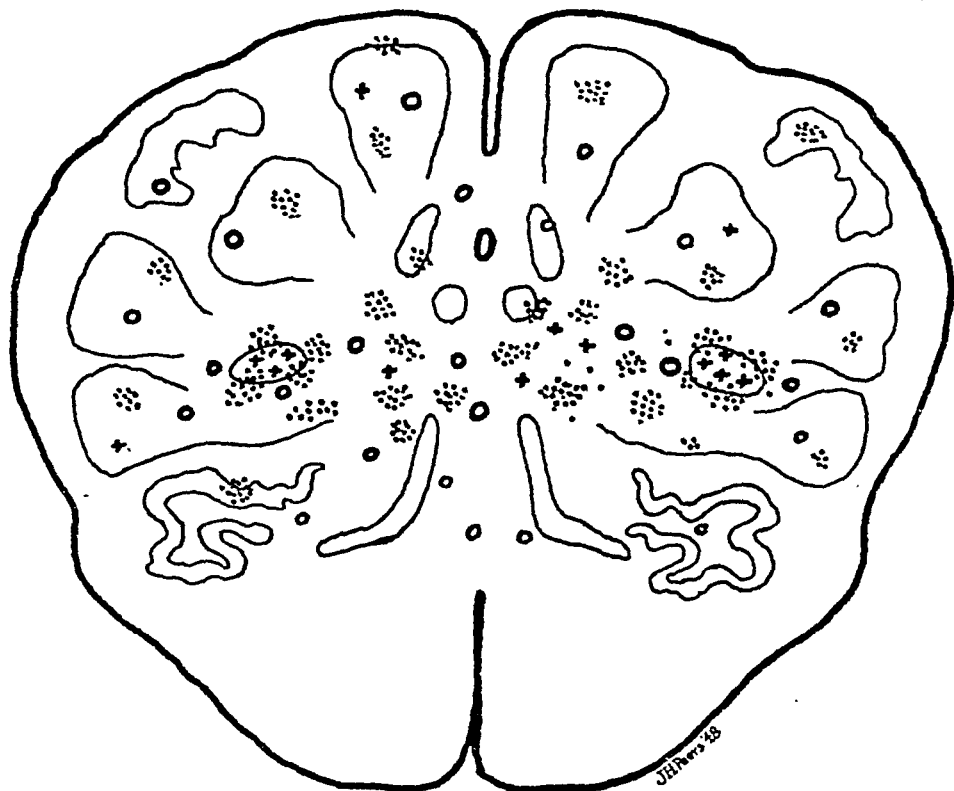


Text-Figure 1. Medulla at decussation of pyramids. In this and subsequent figures, perivascular infiltrations are represented by circles, interstitial infiltrations by groups of dots, and dead cells by crosses. Major nuclear groups are indicated in thin outline.

(Text-Fig. 2) and at the olives (Text-Fig. 3), but was considerably less severe than might have been expected in a nucleus of pure somatic motor neurone type. Some degree of perivascular infiltration, usually sparse, was present in nearly all specimens. Focal microgliosis, also generally of mild degree, was present in about 80 per cent of the sections. On the other hand, necrotic nerve cells were few and were noted in only one-third of the specimens. Such destructive lesions usually were unilateral, and the opposite nucleus often appeared practically normal. Occasionally a small foam cell abscess occupied a portion of the affected nucleus. The observed lesions rarely appeared to have been sufficiently extensive to have caused recognizable paresis of the tongue during life, a fact also noted in clinical descriptions of poliomyelitis. Horányi-Hechst<sup>1</sup> re-

ported a similar light and irregular damage in this motor nucleus. Swan<sup>2</sup> evidently observed greater damage in his small series, since he stated that the ambiguous and hypoglossal nuclei were equally affected.

The *nucleus ambiguus* is a long, irregular column of large nerve cells of motor type lying in the lateral part of the reticular substance and more or less recognizable throughout the length of the medulla. It forms

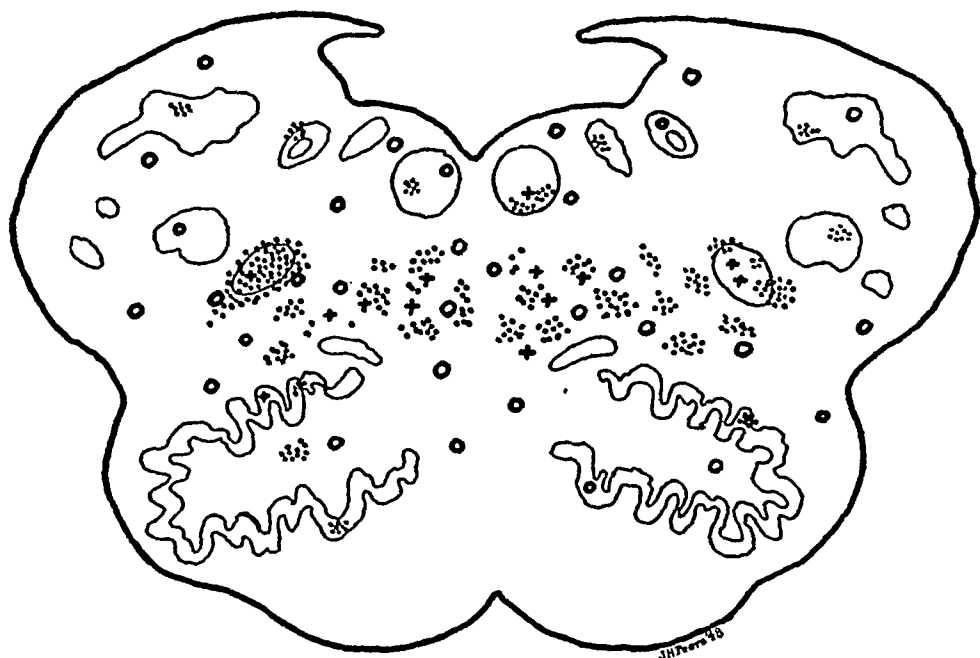


Text-Figure 2. Medulla at the closure of the fourth ventricle.

part of the branchial motor component. Axones from its cells run in the glossopharyngeal, vagus, and accessory nerves to supply the striated musculature of the pharynx and larynx. The nucleus ambiguus was invariably damaged and usually severely in all specimens at all levels (Text-Figs. 1, 2, and 3), as would be expected from the pharyngeal paralysis and obstruction of the airway so common in the terminal stages of fatal poliomyelitis. The severity of the infiltrative lesions appeared to be of about equal degree at the three levels examined. The lesion was graded as moderate in about one-third and severe in two-thirds of the specimens. Necrotic nerve cells were seen in about one-half of the specimens. They were noted somewhat more frequently at the level of the pyramidal decussation, quite likely because at this level the nucleus ambiguus is most poorly defined and other necrotic cells of the upper end of the anterior horns were incorrectly assigned to it. The severity

of the lesions was roughly equal on the two sides in about three-fourths of the specimens, and notably asymmetric in the remaining one-fourth.

At the level of the mid-portion of the olives (Text-Fig. 3), where the nucleus ambiguus is largest and most clearly defined, there was almost complete destruction of one or both nuclei in one-fourth of the specimens. Not only had almost all the nerve cells disappeared, but the ground substance had dissolved and the resulting cavity was filled with



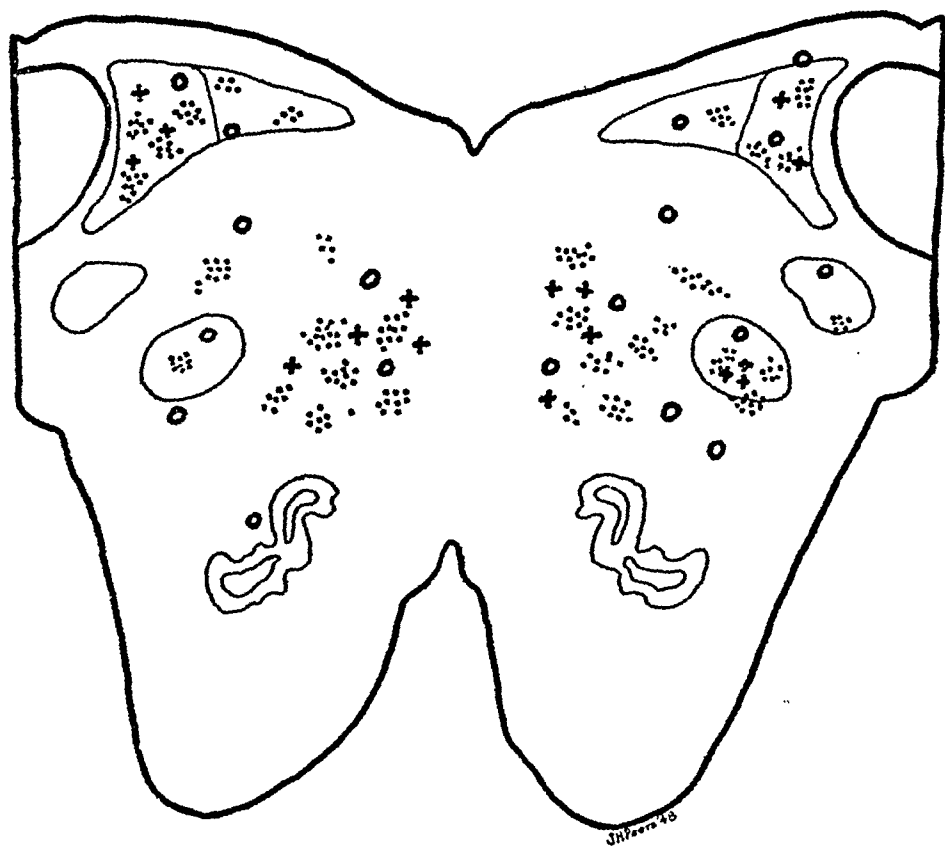
Text-Figure 3. Medulla through the mid-portion of the inferior olives.

a mass of phagocytic microglia with vacuolated cytoplasm filled with lipoid débris. Such foam cell abscesses were unilateral in 6 and bilateral in 4 specimens. Their occurrence could not be correlated with the reported duration of the illness, or the epidemic from which the specimen was obtained.

The *motor nucleus of the facial nerve*, also part of the branchial motor component, is an oval group of large cells of motor type lying on the border between the pons and medulla, in a ventrolateral position corresponding to that of the more caudal nucleus ambiguus. Its cells send motor fibers to all of the small muscles of the face, the stylohyoid and the posterior belly of the digastric muscle. In our material the facial nuclei never entirely escaped injury (Text-Fig. 4). There was some degree of perivascular infiltration, usually scanty, in all specimens. Foci of microgliosis were present in all but 2 specimens. When divided according to the abundance of this interstitial reaction, one-third were recorded as mild, one-third moderate, and the remaining one-third as marked.



One or two necrotic nerve cells were observed in one-half the specimens. In an additional one-fourth, dead cells were moderately numerous. In the remaining one-fourth no cells were recorded as actually necrotic. In the majority of cases the lesions seemed roughly symmetric on the two sides, but occasionally one nucleus was severely injured while the opposite presented only minimal inflammatory reaction. Both Horányi-Hechst<sup>1</sup> and Swan<sup>2</sup> recorded briefly essentially similar observations.



Text-Figure 4. Medulla at the emergence of the eighth nerve roots.

There was no satisfactory correlation between the amount of damage observed in the facial nuclei in sections and the reported occurrence of facial paralysis in the available clinical histories. Sections from patients stated to have had facial paralysis showed severe histologic lesions in the facial nuclei, often on the corresponding side, but many other specimens presented apparently equally severe lesions although the available history made no mention of facial paralysis. In only one instance did the section from a patient having facial paralysis during life fail to present significant lesions in the facial nuclei. This excess of lesions in the facial nuclei over clinically observed facial paralysis may be due in part to the likelihood that lesser degrees of facial paresis passed unnoticed in the critically ill or moribund patients. No lesion observed was so extensive as totally to destroy the nuclei in section, and

in most instances there probably were sufficient functioning neurones left to prevent marked or complete facial paralysis.

The *reticular substance* is an irregularly shaped mass of gray matter in the center of the medulla, filling the interspaces between the large fiber tracts and discrete nuclei. It extends through the entire length of the medulla. It is composed of small and medium-sized nerve cells among which are a number of large cells of motor type, especially in the more anterior portion. This formation appears to be a relay center for many reflexes tending to maintain the body in static position. Vomiting and swallowing reflexes are also mediated through it. Of especial interest is the fact that the seat of control of the rhythm of respiration appears to be located in its more caudal portion.

As might be expected in a group of fatal cases of poliomyelitis, chiefly of the bulbar type, the reticular substance presented marked lesions in all specimens and at all levels (Text-Figs. 1 to 4). Both perivascular and interstitial infiltration were pronounced. The severity of the damage increased notably in the more anterior levels near the pons, and the necrotic nerve cells were most numerous in the anterior giant-celled portion having connections with the vestibular nuclei. In addition, comparison with control sections of normal brain suggested that up to one-half of the large cells had disappeared without leaving any trace behind. Both Horányi-Hechst<sup>1</sup> and Swan<sup>2</sup> reported essentially the same lesions in their specimens. Correlation of these lesions with signs and symptoms in the living patient was virtually impossible. The majority of patients on admission were critically ill and severely prostrated and static positional reflexes for obvious reasons were not examined and recorded. The majority of patients died of respiratory failure, but most often the available records did not distinguish between paralysis of the muscles of respiration and collapse of the central regulatory mechanism. In five cases, however, the records mentioned jerky and irregular respiration with pauses and marked variation in rate, and in all of these patients lesions were pronounced in the posterior end of the reticular substance.

The *dorsal motor nucleus of the vagus*, principal representative of the visceral motor component in the medulla, is a long slender group of small spindle-shaped cells lying beneath the floor of the fourth ventricle somewhat lateral to the mid-line. Axones from its cells pass through the vagus nerve to the ganglia of the thoracic and abdominal sympathetic plexuses. There they synapse with secondary neurones innervating the heart and a wide variety of smooth muscle and glands in the respiratory and digestive tracts. It is thus the central efferent nucleus of the widely distributed autonomic nervous system. Unlike the peripheral

portions of the sympathetic system which rarely show lesions in poliomyelitis, this central nucleus frequently exhibited some inflammatory reaction, although such changes were seldom marked. One or two perivascular collars of lymphoid cells were observed in about 80 per cent of specimens at all three levels, but it is doubtful how many of these represented reaction to toxic material originating in deeper structures and oozing out along emergent vessels traversing the nucleus. In about one-half of the specimens definite parenchymatous lesions in the form of small foci of microgliosis were present at the level of the pyramidal decussation (Text-Fig. 1) and at the ventricular closure (Text-Fig. 2). At the level of the inferior olives, where the nuclei are larger and more distinct (Text-Fig. 3), such focal lesions were seen in only one-third of the specimens. No necrotic nerve cells were noted in any specimen at any level. Both Horányi-Hechst<sup>1</sup> and Swan<sup>2</sup> made brief mention of slight to moderate involvement of the dorsal vagus nuclei in about one-third of their specimens.

The *nucleus of the tractus solitarius*, visceral sensory component in the medulla, is an eccentric mantle of gray matter wrapped around the rostral end of the descending tractus solitarius much like cotton about an applicator stick. It consists of small nerve cells of sensory type on which terminate the incoming fibers of the facial, glossopharyngeal, and vagus nerves. The anterior part is concerned with the sense of taste while more caudal portions are part of the reflex mechanisms of swallowing, respiration, and vomiting. At the level of the ventricular closure, lesions were uniformly mild but frequent. Scanty perivascular infiltration was noted in almost all specimens, while slight focal microgliosis was present in slightly over two-thirds. No necrotic cells were seen. In 2 specimens one nucleus was intact and in one both nuclei were free of lesions at this level.

More anteriorly, at the level of the olives (Text-Fig. 3), damage in the nucleus solitarius was somewhat less frequent and noticeably more uneven in severity. In 5 specimens both nuclei were intact, and in 7 more, one nucleus was free of lesions. In the remainder, slightly over two-thirds of the series, there was both perivascular infiltration and focal microgliosis. Usually this was of moderate degree, but in 3 cases it was recorded as marked and in one, single necrotic nerve cells were seen. It is worthy of note that in the three specimens showing severe lesions in the solitarius, the dorsal motor vagus and the ventral motor (ambiguus) nuclei were also severely damaged. However, the converse was not true and severe injury was commonly noted in the ventral motor vagal (ambiguus) nuclei unaccompanied by correspondingly marked lesions

in the nucleus solitarius. This nucleus is not specifically mentioned by either Horányi-Hechst<sup>1</sup> or Swan.<sup>2</sup>

The *nucleus of the spinal tract of the trigeminal nerve* forms a rather large bilateral mass of small and some medium-sized nerve cells scattered sparsely in a fine-grained fibrillar matrix. This nucleus, a continuation of the substantia gelatinosa capping the posterior horns, extends through the medulla to the mid-pons, but at higher levels it is less distinctly marked out. It and the remaining nuclei described below form the somatic sensory component of the medulla. Clinically apparent alterations in its function as first nucleus of the sensory portion of the trigeminal nerve was hardly to be expected. However, as sensory nucleus for the mucous membranes of the mouth and nose, lesions in it are of considerable interest in relation to these regions as possible portals of entry of the virus.

At the most caudal of the three levels studied—that through the pyramidal decussation (Text-Fig. 1)—at least a mild degree of perivascular infiltration appeared in all but one specimen. In the absence of parenchymatous lesions in the nuclei, the significance of these is uncertain since many of the vessels supply deeper and more severely damaged regions of the medulla and traverse the rather bulky spinal trigeminal nucleus in reaching the meningeal surface. Hence the milder degree of perivascular infiltration may be only the cellular reaction to effusion of toxic products of degeneration from the severely damaged deep structures. Parenchymatous lesions in the form of diffuse and focal microglial proliferations were noted in the spinal trigeminal nuclei of both sides in about one-half the specimens. Usually they were of unequal severity. In one-fourth of the specimens such lesions appeared on only one side—perhaps accidentally, chiefly on the right. In the remaining one-fourth no parenchymatous lesions were seen in sections at this level. In the large majority of specimens with lesions there was only mild microglial proliferation with one or two small focal accumulations. The majority, although not all, of these lesions were located in the inner or spongy zone of the nucleus. In about 10 per cent of the specimens focal and diffuse microgliosis was quite marked and occurred not only in the gray matter of the nucleus but also in irregular, usually perivascular patches in the white matter of the descending spinal tract of the trigeminal nerve lying external to the nucleus. Very rarely a single necrotic cell was seen.

At the level of the ventricular closure (Text-Fig. 2), parenchymatous lesions in the spinal trigeminal nuclei were almost identical in frequency and severity with those at the level of the pyramidal decussation.

The upper portion of the spinal trigeminal nucleus appearing at the

level of the olives (Text-Fig. 3) presented considerably less damage than was noted at the lower levels. Parenchymatous lesions were only about one-half as frequent. One-third of the specimens showed a few small foci of microgliosis on both sides; in one-sixth, lesions were unilateral, while the remaining one-half presented no parenchymatous lesions. Only 2 specimens ran clearly counter to the general trend for damage to diminish rostrally. In both of these, lesions in deeper structures—ambiguus and reticular substance—were unusually severe at this level. In practically all specimens there was some degree of perivascular round-celled infiltration, although it was usually somewhat less marked than at lower levels in spite of the usually severe and extensive lesions in the deeper structures. Swan<sup>2</sup> made note of the occasional occurrence of focal infiltrations in the spinal trigeminal nucleus, but saw no necrotic nerve cells, probably because of the limited number of specimens available to him. In contrast, Horányi-Hechst<sup>1</sup> stated categorically that this nucleus remained entirely free of histologic alterations in every case. No explanation for this discrepancy is apparent.

The *nucleus gracilis* is the first relay station in the long dorsal column pathway. Axones from the spinal ganglia of the lumbosacral segments bearing proprioceptive sensation from the lower limbs ascend in the funiculus gracilis and end on the cells of the nucleus gracilis. Axones of the cells of the nucleus gracilis cross to the opposite side in the sensory decussation and ascend in the medial lemniscus to the ventral nuclei of the thalamus. In about 80 per cent of the specimens some degree of perivascular infiltration, usually bilateral and of mild degree, was present (Text-Figs. 1 and 2). Definite local damage in the form of focal and diffuse microgliosis was somewhat less frequent. About three-fourths of the specimens showed such lesions. Of these, somewhat less than one-half showed only one or two small foci on one side only. In the remainder, scattered foci of reactive microglia were present on both sides. In about 20 per cent of the specimens, one or two necrotic nerve cells were noted in addition to the inflammatory infiltration. In only 3 specimens, or about 7 per cent of the total series, was the nucleus gracilis found to be entirely free of inflammatory reaction.

The *nucleus cuneatus* is very similar in structure and connections to the nucleus gracilis, but its incoming fibers have their origin in the ganglia of the upper thoracic and cervical segments, and carry proprioceptive sensation from the arms and the neck. The axones of its cells run upward along with those from the nucleus gracilis in the medial lemniscus and end in the ventral thalamic nuclei. In the series of specimens as a whole, lesions in this nucleus were identical in number and

intensity with those in the nucleus gracilis (Text-Figs. 1 and 2). In individual specimens also, the lesions were almost always of nearly equal degree in the two nuclei. In no case was one nucleus spared when the other was severely damaged. Both Horányi-Hechst<sup>1</sup> and Swan<sup>2</sup> appear to have observed a corresponding amount of damage in these nuclei of the long dorsal tracts.

The *lateral cuneate nucleus*, in spite of its name and location, is not a part of the cuneate fasciculus and nucleus. Rather it is the analog in the medulla of Clarke's column in the cord. To its cells are brought tone impulses from muscle, joint, and tendon receptors in the neck and possibly in the upper limb. Axones from cells of the lateral cuneate nucleus pass via the restiform body to the vermis, especially to its anterior part. At the level of the ventricular closure the lateral cuneate nuclei present some degree of perivascular infiltration—usually scanty, and bilateral in about three-fourths of the specimens (Text-Fig. 2). Interstitial focal microgliosis was present in about 40 per cent of the whole series. In one-third of the specimens showing parenchymatous lesions, the focal infiltrations were few and present on only one side. In slightly more than one-fifth the nucleus appeared entirely normal at this level.

Farther rostral, at the level of the olives (Text-Fig. 3), the lateral cuneate nuclei presented more frequent and somewhat more severe damage. In all but 2 specimens examined there was some degree of perivascular infiltration, generally small in amount and almost always bilateral. Interstitial microgliosis was noted in about one-half the specimens. Unilateral and bilateral parenchymatous lesions were about equally frequent. Single necrotic nerve cells in one nucleus or the other were observed in about 15 per cent of the specimens examined. In only two instances were there no visible lesions. Horányi-Hechst<sup>1</sup> and Swan<sup>2</sup> do not specifically mention the lateral cuneate nuclei.

The *inferior olives* form a large and conspicuous nuclear mass extending through most of the length of the medulla. The olives are sensory nuclei by embryologic derivation and function, being a relay station, en route from the brain stem and cervical cord to the cerebellum, for impulses coordinating movements of the eyes, head, and upper limbs. The course of the afferent fibers to the olives is not known with certainty, but the axones of the olivary cells cross the midline to join the restiform body and to be distributed widely to the cerebellar cortex of the opposite side. Those from the superior and medial accessory olives go to the older midline portion of the cerebellar cortex, while those from the ventrolateral part of the main nucleus have been traced to the more recently developed cerebellar hemispheres. A few fibers are believed to descend

in the olive-spinal tracts of Helweg to the anterior horn cells of the upper segments of the cervical cord.

As was the case with the large sensory nuclei previously described, perivascular round-celled infiltration of slight to moderate degree was observed in the inferior olives of practically every specimen in the series. Only 2 were noted as being entirely free of lesions of any sort, and in each case the olives were normal only in the caudal section through the closure of the ventricle. A number of these perivascular infiltrations doubtless did not represent damage in the olivary nuclei but rather reaction to drainage of toxic products along radiating vessels from the overlying severely injured reticular substance.

However, parenchymatous lesions indicating direct injury to the olivary nuclei were observed in 27 per cent of the specimens. They occurred with nearly equal frequency in sections through the middle of the olives (Text-Fig. 3) and in the posterior part of the nuclei at the level of the ventricular closure (Text-Fig. 2). For the most part the lesion consisted of single loose foci of proliferating microglia, almost always on one side only. Rarely, single foci were found on both sides. Many of these foci of microgliosis seemed to represent response to a direct injurious action of the virus on the ground substance rather than proliferation secondary to degeneration and necrosis of a nerve cell. The foci were rather large and loose. The regular pattern of nerve cells in the olivary nucleus was not disturbed and nearby nerve cells showed no morphologic evidence of degeneration. One such focus was found among the white fibers of the hilum of the olive where no nerve cells are present. However, in 6 specimens there was actual necrosis of one or two nerve cells, with neuronophagia in the form of small, tight clusters of microglia in addition to the larger and loose interstitial foci not visibly related to nerve cell degeneration. Horányi-Hechst<sup>1</sup> briefly noted the occurrence of slight lesions in the olives in single cases in his material. Swan<sup>2</sup> reported the frequent occurrence of perivascular infiltration in the hilum, but saw no parenchymatous lesions in the olives in the limited number of specimens in his series.

The spatial arrangement of these parenchymatous lesions in the contour of the olivary nucleus was unexpected and perhaps significant. If the virus had entered the olivary nucleus by simple seepage from the overlying severely damaged nucleus ambiguus and reticular substance, it would have been expected that lesions would have tended to be concentrated in the dorsal portion of the olive nearest the source of the virus. If the virus penetrated the side of certain axones of the olivary cells as they traversed the restiform body, a random distribution of

lesions throughout the olivary nuclei would have seemed most likely. In fact, neither pattern of lesions appeared in the specimens. Instead, the glial foci were quite clearly concentrated in the medial half of the olivary nucleus, and about equally distributed between the dorsal and ventral plates of the nuclear complex. This medial portion of the olive is connected with the midline areas of the cerebellum which is the only portion of the cerebellum where lesions are found regularly. Such a concentration of lesions in the two interconnected portions of what otherwise appear to be internally homogeneous brain areas is noteworthy.

### SUMMARY AND CONCLUSIONS

In a study of the anatomic distribution of the lesions of acute poliomyelitis in 42 specimens of the medulla oblongata it was found that lesions of maximum severity are regularly encountered in the reticular substance, nucleus ambiguus, and supraspinal and spinal accessory nuclei. Other somatic and branchial motor nuclei unaccountably present much less severe and constant lesions.

Nuclei of the sensory components frequently contain a few small lesions. Such lesions are somewhat more numerous and severe in the somatic than in the visceral sensory nuclei. The occurrence of single necrotic nerve cells with typical reaction in each of these nuclei in a few specimens indicates that the cells of the sensory nuclei are not completely refractory to the virus of poliomyelitis.

While a few individual specimens presented moderate quantitative differences from the average pattern of lesions, the series as a whole gives a general impression of uniformity. Within the series no grouping is discernible that suggests an essential difference of pathogenicity of supposedly various strains of virus. Also, there are no consistent differences in disease pattern corresponding to the stated duration of illness, or to whether it began with bulbar or spinal symptoms. We are obliged to conclude that death in poliomyelitis occurs when a certain and fairly constant amount of damage is produced in the medulla, and that the final pathologic end-result, in the medulla at least, is relatively uniform, and apparently independent of the route of spread of the virus, or of the speed with which the vital centers are overwhelmed and disorganized.

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# THE RECOVERY OF SKELETAL MUSCLE FIBERS FROM ACUTE ISCHEMIA AS DETERMINED BY HISTOLOGIC AND CHEMICAL METHODS\*

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In a previous study of the histologic changes induced in skeletal muscle by acute ischemia,<sup>1</sup> it was found that death of muscle fibers occurred in an orderly, progressive manner up to 12 hours, at which time the extent of necrosis is considerable. Subsequently it was shown<sup>2</sup> that, even following brief intervals of ischemia, further extension of the necrosis was not prevented when a vascular occlusion was released. The duration of ischemia was perpetuated by both stasis and capillary damage, thereby increasing significantly the extent of necrosis. Although the increase in necrotic fibers was marked, many appeared normal by histologic standards after as long as 6 hours of complete ischemia. The fate of such intact fibers is of paramount importance; their number and their ability to regain contractility, among other factors, may determine the degree of functional recovery of the muscle as a whole.

It has been tacitly assumed<sup>3,4</sup> that, with the initiation of ischemia in skeletal muscle, there is a *pari passu* depletion of energy reserves, onset of autolysis, and incidence of death in all the muscle fibers. Depletion of energy reserves is presumed to be a criterion of cell death<sup>5</sup> and in some instances of autolysis.<sup>6</sup> While current studies of chemical events appear to substantiate such a view, the behavior of the histologic pattern is not so easily reconciled with it, unless there are within many fibers alterations more subtle than are detectable by microscopic examination. Because of this discrepancy it is relevant to ascertain the ability of such fibers to recover from ischemia and to determine the factors responsible for cell death or irreversible changes in fibers; it is particularly important to decide whether the essential irreversible event is structural or chemical. If chemical alteration alone is the determinant, the histologic picture may be equivocal in the evaluation of viability; whereas if the histologic structure represents a reliable criterion of viability, its exact significance deserves closer consideration. This relationship of histologic structure to viability and chemical change is, therefore, the object of the present study.

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## EXPERIMENTAL PROCEDURES

Ischemia was produced in one hind extremity of male albino rabbits, weighing 2000 to 3000 gm. and of Sprague-Dawley male rats, weighing 250 to 300 gm., by a technic previously described.<sup>2</sup> After varied periods of ischemia the tourniquet was released and the extensor muscles were excised during nembutal narcosis from both the ischemic and normal legs, following recovery for 0, 3, and 24 hours. Immediately before excision the muscles were tested for contractibility by faradic current.<sup>1</sup> The excised muscles were fixed in 10 per cent formalin, at 37° C., embedded in paraffin, and sectioned 8  $\mu$  thick. The muscles were sampled to allow representative examination of all parts; sections were stained with hematoxylin and eosin. In each section the normal and necrotic fibers were identified, and 100 consecutive fibers were counted differentially, so that an average percentage of normal and of diseased fibers was calculated for each muscle. The contralateral muscle from the normal limb served as a control in each animal.

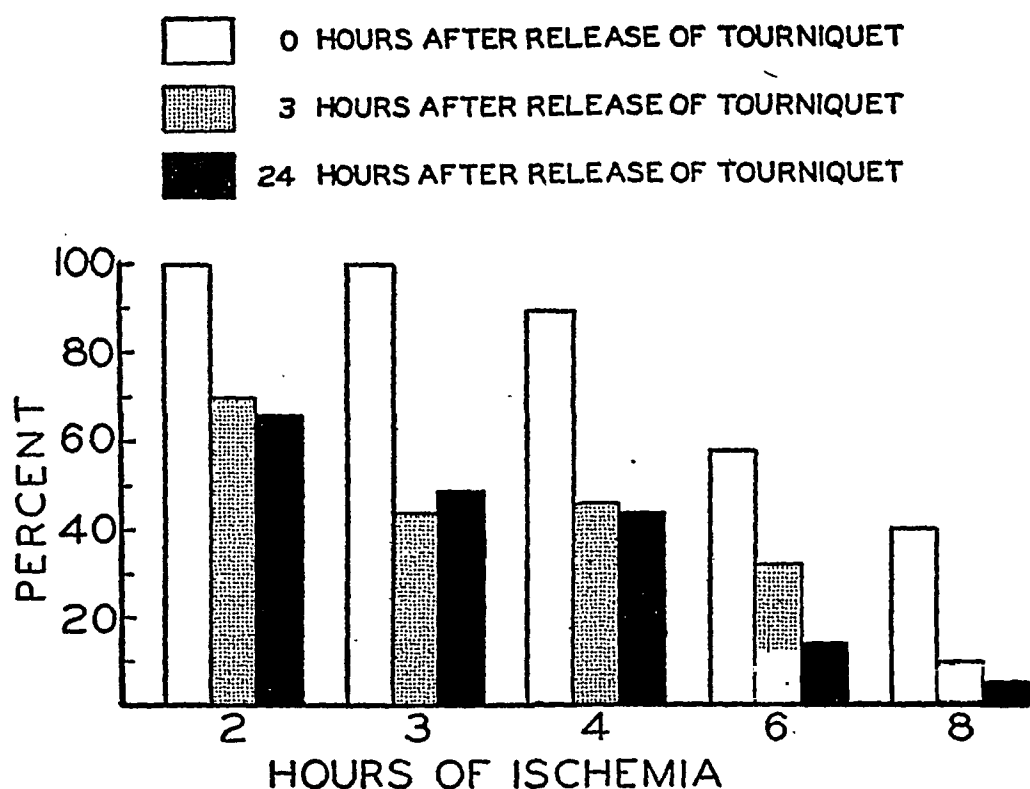
In a second series of male rats the tourniquet was applied to one thigh for 4 hours and a recovery period of 24 hours permitted, after which the animals were narcotized with nembutal and the extensor muscles of the legs exposed. The distal tendinous attachment of the tibialis anticus muscle was carefully sectioned and the muscle freed by gentle dissection to its proximal fleshy attachment. Contractility was tested by a faradic current and sufficient time for restitution then allowed, after which the muscle was rapidly excised and plunged into liquid air. The frozen muscle was then pulverized in a chilled bronze chamber. The chamber consisted of a central well into which was fitted a closely adapted solid cylinder for crushing the tissue, with a surrounding moat for containing the chilling liquid, usually liquid air. Portions of crushed tissue were distributed to tared 50 cc. flasks containing 5 cc. of cooled 10 per cent trichloroacetic acid and zinc sulfate solutions, respectively. After reweighing the flasks the tissues were examined for several components. The fraction contained in trichloroacetic acid was exhaustively extracted and the extract analyzed quantitatively for adenosine triphosphate, phosphocreatine, and acid-soluble phosphorus by the scheme of LePage.<sup>7</sup> Glycogen was estimated on the extracted residue by the method of Good, Kramer, and Somogyi,<sup>8</sup> with determination of sugar by adaption of Hoffman's<sup>9</sup> technic to a spectrophotometric procedure. The fraction extracted by zinc sulfate solution was used for determination of lactic acid by Winnick's microdiffusion method.<sup>10</sup>

The analytic figures were corrected for increases in volume due to edema by simultaneous excision of the digitorum longus muscles from

the ischemic and normal legs. These muscles were weighed in tared crystal dishes at once and were reweighed after drying for 12 hours at 110° C. Corrected values might have been expressed by MacFarlane and Spooner's formula<sup>11</sup> or, as in this instance, interpreted directly for 100 gm. of dried tissue. It is assumed that there is no significant loss of tissue protein from the damaged muscle.

### RESULTS

With complete unrelieved ischemia the histologic pattern changed from the normal syncytoid<sup>1</sup> structure at the second hour, with disappearance of the normally conspicuous longitudinal striations and accentuation of the transverse striations, which were much less conspicuous in



Text-Figure 1. The percentage of histologically normal fibers after different periods of ischemia and intervals of release for 65 rabbits.

the rapidly fixed muscle. Further ischemia was associated with the appearance of Bowman's discoid degeneration (Fig. 1) at about the fourth hour; the number of fibers affected by this change reached a maximum of nearly 90 per cent by the twelfth hour of ischemia. If the ischemia was relieved at any time after the second hour, there was a further extension of degenerated fibers over the next 3 hours, when the number reached a maximum and did not increase even after 24 hours of recovery (Text-Fig. 1). It was apparent, however, that, in contradistinction to the muscles with unrelieved ischemia, those with release of

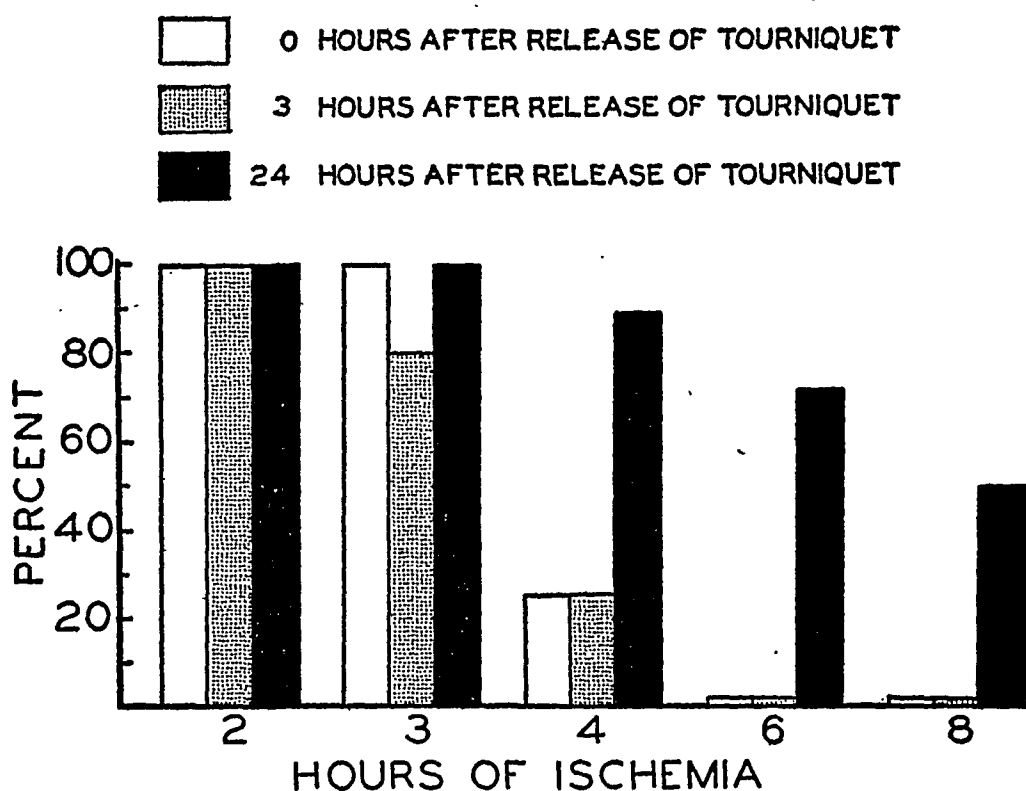
occlusion contained fibers with several types of necrosis. Many of the fibers conformed in appearance with Zenker's hyaline degeneration; they were large, homogeneous, and contained a structureless, faintly acidophilic cytoplasm (Fig. 2). Others were composed of a granular, disorganized, deeply acidophilic cytoplasm and might be designated as showing granular necrosis (Fig. 3) in accordance with Fishback's description.<sup>12</sup> The percentage of these different forms of necrosis in the muscles released from ischemia was variable and unpredictable; occasionally the Bowman's type was predominant, and at other times either the Zenker or the Fishback form was most prevalent. It was not possible to discern the transformation of one form to another, except that certain fibers appeared to progress through either the discoid or waxy degeneration to a granular disintegration. It was clear, however, that neither Zenker's degeneration nor Fishback's granular degeneration was to be seen in muscles with unrelieved ischemia; they occurred exclusively in ischemic muscles to which the blood supply was readmitted.

All three forms of degeneration were therefore grouped as necrotic fibers in the fiber counts, because of this independent variability. In Text-Figure 1 it is seen that not until the fourth hour did necrotic fibers appear in completely ischemic muscles; after that time the rate of appearance was progressive until at 8 hours of ischemia only 33 per cent of structurally normal fibers remained. When the tourniquet was released for a period of 3 hours prior to excision of the muscle there was a sudden increase in the necrotic fibers, which was proportional to the duration of ischemia and unrelated to the number seen before release of the obstruction. By the eighth hour of ischemia, only 10 per cent of the fibers were structurally normal (Fig. 4). The extension of the period of release up to 24 hours did not significantly alter the percentage of residual normal fibers, although some decrease was manifest after ischemia of 6 and 8 hours' duration.

It is apparent that release of the occlusion, rather than obviating the necrosis, favors its extension. The return of blood flow is associated with a sudden increase in necrotic fibers, which rapidly reach a maximum with little subsequent progression. The connection between the inflow of blood, the appearance of the two forms (Zenker's and Fishback's types) of degeneration, and the sudden increase in necrotic fibers seem correlative. These phenomena may well be, but in such an instance one would expect that after ischemia for 2 and 3 hours with subsequent release, such muscles would contain necrotic fibers solely of these types, especially as Bowman's disks are absent before release, yet all forms

are usually well represented. Even if the inflow of blood is instrumental in the increase of necrosis, other factors cannot be excluded.

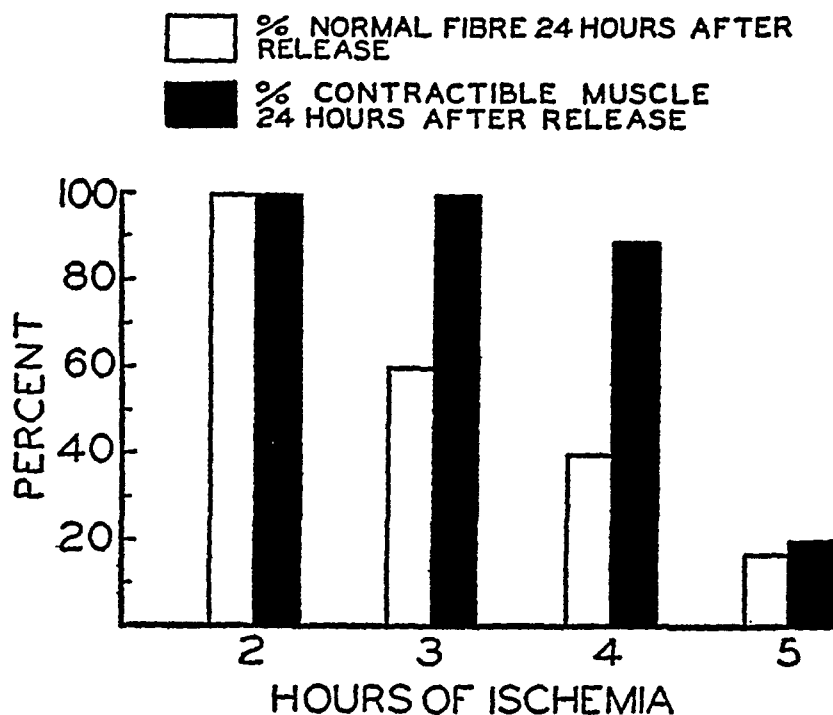
When the contractility of muscles was assessed after various periods of ischemia, it was found that nearly all had lost this property by 4 hours, and all had become non-contractible by six hours (Text-Fig. 2). By 3 hours after release of vascular occlusion the state of the muscle was little altered, whereas by 24 hours after release a considerable number of muscles had regained contractibility to greater or less degree. Up to 90 per cent had recovered marked ability to contract after 4 hours of ischemia, and 50 per cent were again contractible, at least in part, after 8 hours of ischemia. As might be expected from the greater percentage of surviving fibers after shorter durations of ischemia, the muscles sub-



Text-Figure 2. The percentage of contractible muscles after various periods of ischemia and intervals of release for 70 rabbits.

jected to briefer periods of ischemia recovered a greater strength of contraction. The survival of histologically normal fibers after different durations of ischemia was paralleled not only in the recovery of contractibility, but also in the vigor of the contraction, which bore an approximate proportion to the extent of fiber survival. In Text-Figure 3 it is demonstrated that the same relationship between contractibility of muscles and recovery of normal fibers also applies to ischemic rat muscle, which follows a similar trend.

To elucidate more exactly the interrelationship of fiber survival and ability of muscles to contract, the muscles were divided into the contractible and non-contractible groups. In each of these the percentage of histologically normal surviving muscle fibers was determined and the average assessed for each period of ischemia for both rabbit and rat muscle. The results are shown in Text-Figure 4. In all contractible muscles the normal fibers comprised a considerable part of the muscle mass, even after 6 hours of ischemia; the low level of 10 per cent was reached only after 8 hours of vascular occlusion. On the other hand, among the non-contractible muscles the average percentage of normal

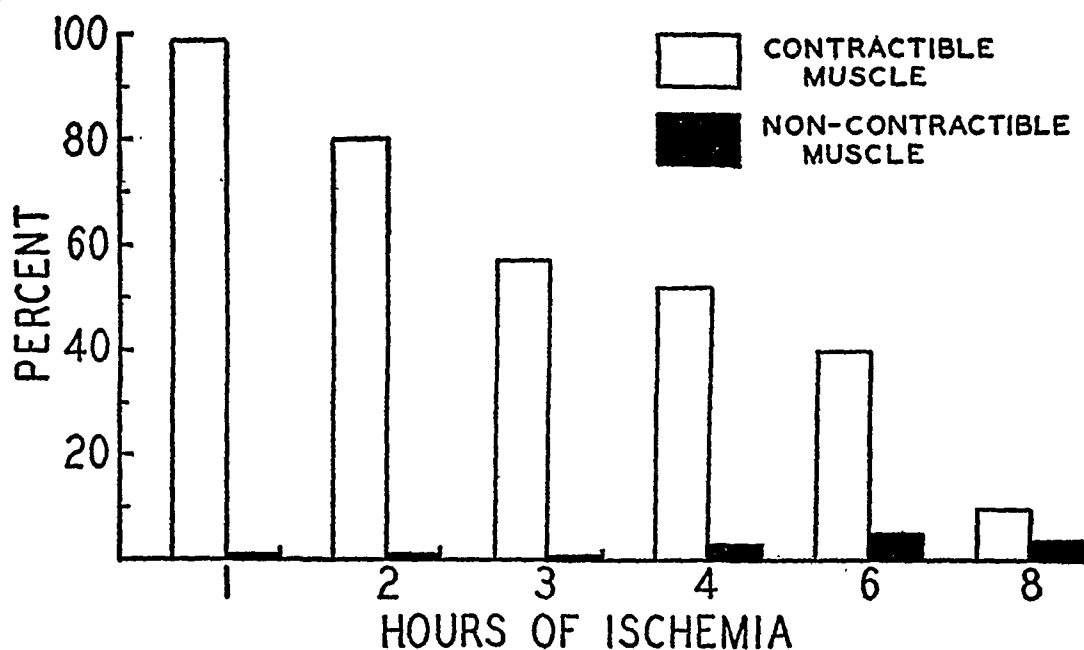


Text-Figure 3. Correlation of percentage of normal fibers and contractible muscles after different periods of ischemia and 24 hours of recovery in 60 rats.

fibers never exceeded 4 per cent; the majority of muscles in this group contained no normal fibers whatsoever. It again became evident that the ability to respond as well as the strength of response to electrical stimulation is intimately related to the presence and number of these histologically normal fibers.

The maximum degeneration of fibers was accomplished within 3 hours after release of the occlusion, whereas contractibility failed to return before about 24 hours subsequent to release despite the presence of many histologically normal fibers. In such instances there existed a form of structurally normal but physiologically inactive fiber. Chemical analyses on ischemic muscle from several rabbits and rats immediately after termination of 4 hours of ischemia revealed that there was

complete depletion of various energy reserves at such time. Such was the condition, also, after a further 3 hours of recovery. However, after a recovery period of at least 24 hours following an ischemia of 4 hours, the restitution of high energy compounds was considerable; adenosine triphosphate, glycogen, and phosphocreatine reaccumulated to between one-quarter and one-third of that found in the contralateral normal muscle (Table I). Whereas in normal muscle such might signify a marked depletion, in this ischemic contractible muscle in which the percentage of histologically normal fibers was 40 per cent or lower (Text-Fig. 3), the re-gain of essential metabolites fairly approximated that of the



Text-Figure 4. Correlation of contractibility and normal fiber percentage at 24 hours after release from different periods of ischemia in 65 rabbits and 60 rats.

surviving contractible tissue. The reaccumulation of these compounds essential to the contractility of muscle accorded well with the structural survival of muscle fibers. Although there was recovery of oxidative metabolic function and resynthesis of both phosphorylated compounds and glycogen, the injured muscles contained a significantly greater amount of lactic acid, indicating the presence of some glycolysis. It is true, however, that these muscles were heavily infiltrated with polymorphonuclear leukocytes to which such an accumulation of lactic acid might be due in part. There also was considerable stasis and sluggishness of circulation which might contribute to the slow removal of the products of contraction induced by the faradic test for contractibility.

The interval required for recovery adequate to permit contraction appears so markedly a function of time that the results of an additional



TABLE I  
Results of Chemical Analyses on Ischemic Rat Muscle \*

Animal no.	Tissue weight	Water	Acid-soluble phosphorus	Phospho-creatine	Adenosine triphosphate†	Lactic acid	Glycogen‡
	gm.	%	mg. %	mg. %	mg. %	mg. %	mg. %
1 Nor.	0.6579	69.6	136.8	243	321	19.5	430.6
1 Isc.	0.7726	82.1	31.2	72	63	23.8	37.6
2 Nor.	0.6212	74.7	123.4	236	296	16.9	414.2
2 Isc.	0.8064	82.2	29.6	62	79	39.5	48.1
3 Nor.	0.8785	71.3	117.5	194	326	20.1	386.7
3 Isc.	0.9729	79.6	28.4	41	120	27.6	56.2
4 Nor.	0.8853	62.4	128.7	233	341	17.8	448.2
4 Isc.	0.9945	89.9	32.4	56	96	37.6	16.2
5 Nor.	0.4064	58.9	121.4	221	352	18.7	426.1
5 Isc.	0.7751	71.2	37.5	49	102	28.4	61.0
6 Nor.	0.3359	67.8	156.0	254	301	22.4	451.0
6 Isc.	0.6383	79.0	22.9	59	51	29.7	
7 Nor.	0.4214	66.8	139.2	216	314	13.6	433.7
7 Isc.	0.5177	77.2	44.1	42	78	23.2	
8 Nor.	0.4133	66.0	119.5	201	329	17.4	424.6
8 Isc.	0.5989	76.5	16.8	21	91		47.2
9 Nor.	0.6081	67.2	124.2	214	311	18.5	419.5
9 Isc.	0.8449	74.6	26.7	68	84	29.6	29.2
10 Nor.	0.6544	75.8	136.6	229	324	22.0	401.2
10 Isc.	0.8216	80.1	31.2	36	81	28.7	102.1
11 Nor.	0.7569	66.3	134.0	231	317	20.6	443.2
11 Isc.	0.8455	77.2	39.7	44	76	34.2	57.1
12 Nor.	0.6423	67.1	127.2	217	312	23.1	432.0
12 Isc.	0.8735	75.4	41.2	31	106	31.2	42.1
Mean: Nor.			130.3	224.0	328.5	19.2	425.9
Isc.			31.0	48.4	85.6	30.3	53.4
Stand.) Nor.			± 10.6	± 16.2	± 17.2	± 2.7	± 18.2
dev.) Isc.			± 7.7	± 14.5	± 17.8	± 4.9	± 19.7
Stand.) Nor.			± 2.2	± 3.3	± 3.5	± 0.5	± 3.7
error) Isc.			± 1.5	± 2.9	± 3.6	± 1.1	± 4.6

\* All rats were subjected for 4 hours to total ischemia of one hind limb and then allowed to recover for a further 24 hours, at termination of which time the chemical analyses were performed on the tibialis anticus muscle. Prior to excision for chemical analysis, the ischemic muscles were tested faradically and were found to be contractile in all animals.

† Calculated from pentose and easily hydrolyzable phosphate (7 min. at 100° C. in HCl).

‡ Glycogen is expressed as glucose.

experiment designed and performed on 25 rats are pertinent. The animals, after 4 hours of ischemia and under nembutal narcosis, were prepared by exposure of the tibialis anticus muscle and attachment of the distal tendon of this muscle to the lever of a kymograph. Tests by direct faradic stimulus elicited no response until at least 21 hours had

elapsed subsequent to relief of ischemia. The factors responsible for this particular time interval of recuperation are not clarified by these experiments and further investigation along different paths will be necessary.

### DISCUSSION

The interpretation of structurally normal muscle fibers, which survive the period of anoxia, as viable cells is substantiated by the recovery of contractibility and resynthesis of energy reserves by muscles containing a significant number of such fibers. Moreover, the ability to contract and the degree of contraction are closely allied to the proportion of histologically normal fibers which persist. Further support of this is offered by the fact that in such muscles as become non-contractible, normal fibers are entirely lacking or present only in small numbers, whereas in contractible muscles these fibers comprise a major element of the tissue. From this it may be deduced that the structural condition of the fibers is an important factor in their ability to recover from ischemia. Fibers which maintain structural integrity throughout the period of vascular occlusion and the first few hours thereafter are with few exceptions capable of regaining function; it is tantamount to saying that structurally intact fibers are viable fibers, although in the early phases of recovery they contain no energy reserves and are still non-contractible.

The view that such fibers, which contain no energy reserves and are non-contractible, are still viable requires elucidation. Definitions of viability depend in one respect upon criteria which are consistent with non-viability or death. If the content of energy-rich compounds and the presence of irritability are accepted as decisive characteristics of living tissue, it might appear that their absence signifies death of the tissue. Upon closer scrutiny it is not entirely logical to conclude that because these attributes signify viability therefore their lack indicates death. It consequently becomes apparent that the state of non-viability and the state of simple depletion of energy reserves may not always be synonymous, although they are usually concomitant. All tissues which suffer exhaustion of high-energy supplies do not *ipso facto* die, even when the duration of depletion is prolonged. Many tissues, muscle particularly, recover their anabolic capacity and regain function. The decision of the incidence of death is related, therefore, not so much to the loss of essential metabolites and function as to the inability of the tissue to regain these within an appropriate length of time. The irreversibility of the loss is the essential factor rather than the loss itself.

In muscle the gap between loss of energy reserves and its irreversibil-

ity is very marked, and for many fibers may represent several hours. If such loss itself represents death or "biochemical death" as it has been called, then all fibers should die simultaneously about the time of exhaustion of these reserves and the cessation of expenditure of energy. We have found this to be untrue for muscle; the fibers commence to die at the time of complete exhaustion of the energy reserve and gradually are destroyed in proportion to the duration of ischemia. The state of the energy reserves, therefore, is indecisive as an indication of the condition of the muscle fibers. This is more accurately reflected in the structural picture of the fibers provided that adequate time is allowed for full development of necrosis, when the histologically normal and abnormal fibers may be accurately separated. Since the structure of the fiber is more closely related to its state of health than is the immediate energy content, it is probable that the decisive determinant of irreversibility is not the condition of energy expenditure but the structural integrity. Although it is acceptable to agree with Schoenheimer<sup>13</sup> that energy must be expended continuously to maintain structure against its tendency to collapse, it may be emphasized that the critical event in the tendency toward irreversibility is the collapse of structure rather than the preliminary breakdown of the energy cycle. The series of events leading to ischemic necrosis may then be enumerated as anoxia, glycolytic depletion of energy reserves, cessation of energy exchange, and structural collapse, which last decides the biochemical irreversibility.

It has been proposed by MacFarlane and Spooner,<sup>11</sup> on the basis of indirect evidence, that ischemic muscle fails to resynthesize phosphorylated adenosine compounds because it is unable to re-amine inosinic acid. But we have found on direct chemical analysis a considerable resynthesis of adenosine triphosphate and phosphocreatine, as well as a marked ability to contract, in muscles recovering from ischemia. The reason for this discrepancy is not readily apparent. It is pertinent, however, that in intact muscle the inosinic acid formed during contraction is rapidly re-aminated,<sup>14</sup> and that this re-amination occurs only under aerobic conditions.<sup>15</sup> The relative anoxia induced by the stasis which follows protracted ischemia<sup>2</sup> may for a time prevent this re-amination, in view of its sensitivity to lack of oxygen. When stasis diminishes and the circulation improves, re-amination takes place. The evidence of our experiments substantiates this view and receives support from the observations of Bollman and Flock<sup>3</sup> that in instances in which fatal shock is avoided the return of acid-soluble phosphates to the ischemic muscle is appreciable after several days, and that after 2 weeks the animals regain functional activity of the ischemic leg.

The observation that following long periods of ischemia many fibers are capable of oxidative resynthesis finds its corollary in the work of Bielschowsky and Green,<sup>16</sup> who postulated that ischemic muscle might be capable of resynthesizing adenosine triphosphate and were able to achieve this *in vitro* with the enzyme systems of both ischemic and normal rat muscle. Our studies have confirmed their results *in vivo* and established the validity of their view that the re-accumulation of the phosphorylated compounds is mainly a matter of adequate recovery time. The enzyme system is present and intact so that other factors may be responsible for the slow rebuilding of the energy reserves. The nature of these factors is not clarified by the present experiments, although there is some indication from the persistent high level of lactic acid that important elements may be stasis and a high state of aerobic glycolysis.<sup>17</sup> The significance of stasis in the perpetuation of ischemia has been discussed in a previous study<sup>2</sup> and is further suggested in the present analysis by the occurrence of Bowman's discoid necrosis in muscles recovering from 2 to 3 hours of ischemia. These forms usually are not found before the fourth hour of ischemia, so that their occurrence in these muscles under the circumstances of recovery indicates some persistent ischemia. In view of the previous vascular experiments<sup>2</sup> the cause of the relative ischemia may be attributed to capillary stasis. It is probable that this persistent stasis and anoxia preclude the accumulation of biochemical reserves and account for the slow restitution of contractility.

#### SUMMARY

The correlation of histologic structure, irritability, and biochemical alterations in skeletal muscles recovering from acute ischemia reveals that histologic evaluation permits a more accurate index of viability of fibers than do the biochemical methods utilized.

Cellular death is not so much dependent upon depletion of biochemical reserves as upon the irreversibility of the depletion. This irreversibility is determined by the integrity of the cell structure.

The slow functional recovery of ischemic skeletal muscle is associated with a prolonged relative ischemia due to stasis, which may delay biochemical restitution in the residual normal fibers.

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## DESCRIPTION OF PLATES

### PLATE II 2

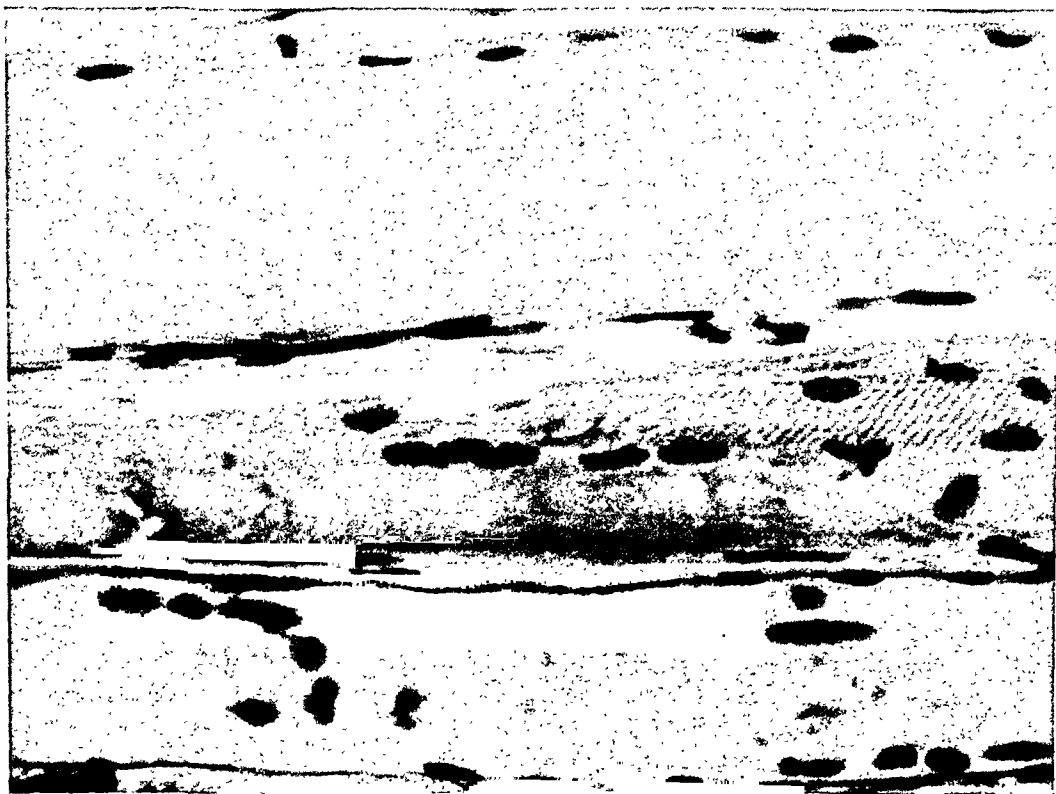
FIG. 1. Muscle fibers from skeletal muscle ischemic for 6 hours followed by a recovery period of 3 hours are composed of Bowman's anisotropic disks. Nuclei are pyknotic. Hematoxylin and eosin stain.  $\times 500$ .

FIG. 2. Zenker's hyaline change is manifest in several fibers removed 3 hours after release from an ischemia of 6 hours' duration. The structureless homogeneity of the cytoplasm and pyknosis of nuclei are evident. Hematoxylin and eosin stain.  $\times 500$ .

1



2



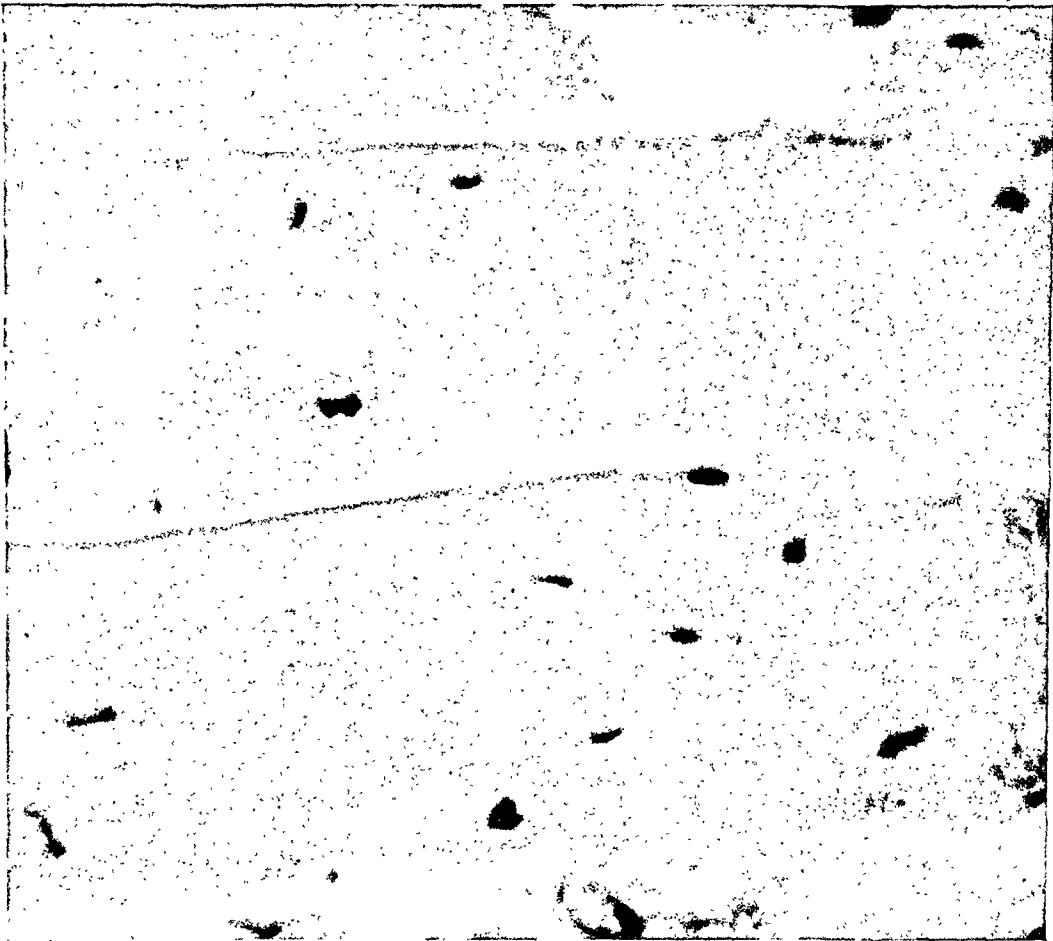
Harman and Gwinn

Recovery of Muscle from Ischemia

PLATE 113

- FIG. 3. The fibers have disintegrated into masses of cytoplasmic granules, a form of granular or "acute molecular" degeneration. The muscle had been released for 3 hours, after 6 hours of ischemia. Hematoxylin and eosin stain.  $\times 500$ .
- FIG. 4. The tissue is an admixture of normal fibers and others in the process of granular or hyaline necrosis. Both edema and acute inflammatory cells are conspicuous. The ischemia lasted 8 hours and was released for 3 hours. Hematoxylin and eosin stain.  $\times 400$ .

3



4







## BLOOD CYSTS ON THE HEART VALVES OF INFANTS\*

T. A. B. BOYD, M.D.

(From the Department of Pathology and Bacteriology, University of Toronto, Toronto, Ont.)

Blood cysts occur frequently on the mitral and tricuspid valve cusps of infants, and rarely on the pulmonary or aortic cusps. They are small, rounded, reddish brown, pin-point to pin-head nodules on the sub-marginal part of the atrial surface of the cusp (Fig. 1), and usually are sessile but may be pedunculated. In different series in the literature, their reported incidence has ranged from 25 per cent (Luschka,<sup>1</sup> 1857) to almost 100 per cent (Fahr,<sup>2</sup> 1906). They have not been found to be related to other congenital anomalies. The cysts seem to disappear with age, as they are uncommon after 6 months of age. Rare cases have been described in children and adults. Elsässer<sup>3</sup> first described these lesions in 1844, and the subject is well reviewed, with complete references, by Levinson and Learner<sup>4</sup> (1932) and Dow and Harper<sup>5</sup> (1936).

Microscopically, they are masses of red blood cells and a few white blood cells packed in a spherical or ovoid cyst, lying in the connective tissue of the cusp, and lined by a single layer of flat endothelium. When large, they project on the atrial surface, and the connective tissue and endothelium of the cusp are stretched over them. They are usually unilocular, but sometimes have small extensions (A, Figs. 2 and 3), or shallow septa.

Their mode of origin is not definitely known, but they have been variously described as hematomas, angiomas, ectatic or dilated blood vessels, regressive blood vessels, and as being due to blood being pressed into crevices on the ventricular surface of the cusp with subsequent fusion of the mouth. All but the last assume that capillaries or blood vessels exist in the cusps, but Harper<sup>6,7</sup> (1940 and 1945) has shown that blood vessels are present only at the base of the cusp.

Several of these cysts were found on the tricuspid and mitral cusps of an infant who had coarctation of the aorta and died 1 hour after delivery. In a study of serial sections of the posterior mitral cusp, two observations were made which supported the last of the explanations given.

Crevices are common along the ventricular surface of the mitral and aortic valve cusps, and are situated between the attachments of the chordae tendinae. Their formation is believed to be aided by the pressure of the blood when the cusps are being fashioned in utero. One of them, (B), is shown in Figures 2 and 3. In Figure 2, the crevice appears to be

\* Received for publication, July 14, 1948.

an empty space, but when followed farther (Fig. 3), it communicates with the ventricle by a narrow mouth. It can readily be imagined how this mouth could undergo closure.

In the blood cysts, evidence that closure had actually occurred is illustrated in Figure 4. Two consecutive sections showed a bar of endothelium joining the ventricular surface to the lining of a small blood cyst.

### SUMMARY

Blood cysts are commonly present on the heart valves of infants. The evidence from serial sections of a mitral cusp supports the explanation that they result from blood being pressed into crevices on the ventricular surface of the cusps. Subsequent fusion of the mouths of the crevices forms the blood cyst.

The photographs were taken by Miss J. G. Brown.

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### DESCRIPTION OF PLATE

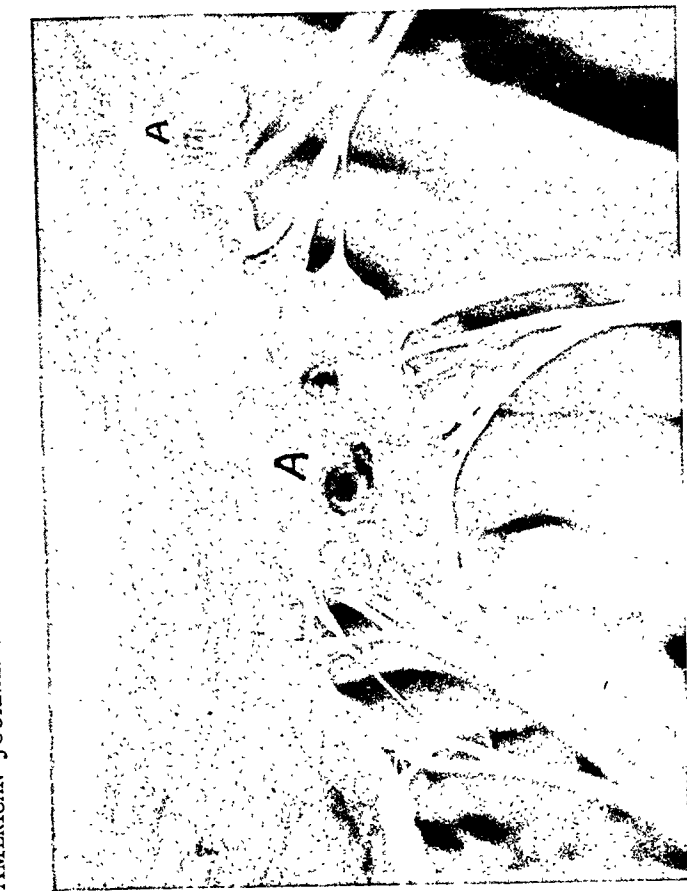
#### PLATE 114

FIG. 1. Blood cysts (A) on the mitral valve. Approximately  $\times 15$ .

FIG. 2. Space (B) represents the crevice. A large blood cyst projects on the atrial surface and two small ones (A) are embedded in the cusp. Hematoxylin and eosin stain.  $\times 65$ .

FIG. 3. Crevice (B) joins the ventricle by a narrow mouth. One small blood cyst is seen to arise as a bud from the other. Hematoxylin and eosin stain.  $\times 65$ .

FIG. 4. A bar of endothelium joins the lining of the small cyst to the ventricular surface of the cusp. Hematoxylin and eosin stain.  $\times 328$ .



1



2



3



4



FORTY-SIXTH ANNUAL MEETING  
OF THE  
AMERICAN ASSOCIATION OF PATHOLOGISTS  
AND BACTERIOLOGISTS

BOSTON

APRIL FIFTEENTH AND SIXTEENTH, 1949



# THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Forty-Sixth Annual Meeting

Harvard Medical School, Boston, Massachusetts

April Fifteenth and Sixteenth, 1949

PRESIDENT GOODPASTURE IN THE CHAIR

## BUSINESS MEETING

April Fifteenth, 1949

Upon nomination of the Council, the Association elected the following officers:

<i>President</i>	SHIELDS WARREN
<i>Vice-President</i>	TRACY B. MALLORY
<i>Secretary</i>	ALAN R. MORITZ
<i>Treasurer</i>	SIDNEY FARBER
<i>Incoming Member of Council</i>	JAMES B. McNAUGHT

For the council, the President announced the following actions:

### Election of new members

A. J. Blanchard, Toronto	Thomas G. Morrione, New York City
George Brecher, Bethesda	Conrad L. Pirani, Chicago
John R. Carter, Iowa City	Lelland J. Rather, San Francisco
William J. Cromartie, McKinney, Texas	Arnold J. Rawson, Philadelphia
David D. Dahlin, Rochester, Minn.	John D. Reese, McCain, N.C.
James R. Dawson, Jr., Nashville	Robert J. Ritterhoff, Cincinnati
Frank R. Dutra, Cincinnati	S. Stanley Schneierson, New York City
Oscar Felsenfeld, Chicago	William E. Smith, New York City
Russell S. Fisher, Boston	Sheldon C. Sommers, Boston
John H. Fodden, Halifax	Sophie Spitz, New York City
John P. Fox, New York City	William H. Sternberg, New Orleans
David G. Freiman, Boston	Herbert C. Stoerk, Rahway, N.J.
Sidney A. Gladstone, New York City	Lotte Strauss, New York City
Abner Golden, Atlanta	Benjamin B. Wells, Houston
John W. Harman, Madison	C. Merrill Whorton, Atlanta
Maurice Lev, Chicago	Irving Zeidman, Philadelphia



Confirmation of a postal vote to reinstate to membership, at his own request, Dr. Charles Oberling.

Acceptance, with regret, of the resignations of Drs. T. S. Cullen, M. S. Fleisher, Russell L. Haden, N. Paul Hudson, Robert Kinnicutt, Mary B. Kirkbride, O. W. H. Mitchell, Phillips Thygeson, and Mary W. Wheeler.

With deep regret, the recording of the deaths of Drs. R. S. Austin, Louis Berger, G. H. Hansmann, George J. Kastlin, Anatole Kolodny, and Robert Koritschoner.

The re-election of Dr. Malcolm H. Soule as Assistant Editor of *The American Journal of Pathology* for the ensuing year, and the re-election of Dr. Shields Warren to the Editorial Board for a period of six years.

The President announced receipt by the Council of a letter from Dr. Karsner in which he expressed the wish to retire from the office of Secretary of the Association. The Council, after serious consideration and with deep regret, had voted to accept Dr. Karsner's resignation from this office. Dr. Sidney Farber read from the floor a resolution of gratitude to Dr. Karsner, and it was voted to spread upon the minutes of this meeting the following resolution, as presented by Dr. Farber:

"The American Association of Pathologists and Bacteriologists has with great regret learned of the desire of Dr. Howard T. Karsner to be relieved of the duties of Secretary because of the assumption of a new position. Out of respect for the expressed wish of one who has done so much for the Association, this wish is accepted. In so doing, the members of the Association make public acknowledgment of the contribution of Dr. Karsner to the very existence of the Association and extend to him our thanks. It is hoped that Dr. Karsner will continue to give the Association the benefit of his wise counsel and advice and that he will remain active in the field of pathology for many years."

The President announced that the next meeting of the Association will be held in Madison. The dates of the meeting will be decided later and will be published in *The American Journal of Pathology* when decision is made. The topic for the symposium for the 1950 meeting is "Reactive Abnormalities of the Vascular and Supporting Tissues with Special Reference to the Collagen Diseases." Dr. Paul Klemperer has been appointed to act as referee.

## REPORT OF THE TREASURER

The report of the Treasurer was submitted to the Council and accepted. It was accompanied by a certification from C. A. Estes, Public Accountant, Boston. In condensed form, the Treasurer's report follows:

## General Checking Account

*Receipts*

Balance on hand, January 1, 1948.....	\$ 2,183.13	
Membership dues:		
Current year .....	\$ 7,550.00	
Previous year .....	70.00	
In advance (1949).....	10.00	
Interest on bonds, from investment account.....	500.00	
		<hr/>
		8,130.00
		<hr/>
		\$10,313.13

*Disbursements*

American Journal of Pathology (\$8.00 per member)....	\$ 7,120.00	
C. E. Lennon (Secretary to Dr. Karsner) .....	150.00	
P. A. Glass (Secretary to Dr. Moritz) .....	150.00	
Reporting 1948 meeting .....	140.75	
National Academy of Sciences .....	500.00	
Officers' expenses to meetings.....	252.20	
Auditing services .....	35.00	
Bank charges.....	2.49	
General office expense, secretary, including expense of annual meeting.....	601.91	
General office expense, treasurer.....	51.66	
		<hr/>
		9,004.01
		<hr/>
Balance on hand, December 31, 1948 .....	\$ 1,309.12	

*Investment Account*

Balance, January 1, 1948.....	\$34,313.24	
Interest on bonds, 1948.....	500.00	
Interest from savings banks.....	230.07	
		<hr/>
		\$35,043.31
Transfer to checking account (bond interest).....	500.00	
		<hr/>
Balance, December 31, 1948.....	\$34,543.31	

*Inventory*

U.S. bonds 2½, series G.....	\$20,000.00	
The Provident Institution for Savings.....	4,053.46	
Franklin Savings Bank.....	4,060.20	
Cambridge Savings Bank.....	4,311.55	
National Shawmut Bank.....	2,118.10	
		<hr/>
Total, December 31, 1948.....	\$34,543.31	



## SCIENTIFIC PROCEEDINGS

INFARCTION OF THE MUSCLE BUNDLES OF THE ATRIA AND VENTRICLES OF THE HEART. William B. Wartman and (by invitation) John Souders, Chicago, Ill.\*

CARDIAC HYPERTROPHY IN YOUNG RATS FOLLOWING RENAL LIGATURE: RATE OF DEVELOPMENT AND EFFECT OF SIMULTANEOUS ADRENALECTOMY. Lelland J. Rather (by invitation), San Francisco, Calif.

*Abstract.* In albino rats subjected to the Grollman technic for inducing hypertension (unilateral nephrectomy and ligature around the remaining kidney), cardiac hypertrophy developed with great rapidity. In a series of animals killed in groups at intervals of 2 to 120 days postoperatively, a statistically significant increase in heart weight appeared as early as 2 days and one-half of the ultimate increase in heart weight was reached in 10 days. No significant progression of hypertrophy occurred after 40 days. Three of 8 surviving rats at 120 days had developed fibrinoid arteritis of the periarteritis nodosa type, involving extrarenal vessels of 200 to 1000  $\mu$  in diameter. None of the animals developed glomerular sclerosis or necrotizing arteriolitis of the type seen in malignant hypertension. Control series of sham-operated and unilaterally nephrectomized rats were used. Comparably prepared animals subjected also to bilateral adrenalectomy showed significant cardiac hypertrophy at 10 days but at 20 days the hearts of these animals were only slightly larger than those of control animals. It is suggested that in the adrenalectomized animals the hypertensive load was not maintained.

THE OCCURRENCE OF ARTERIOSCLEROTIC LESIONS IN PYRIDOXINE-DEFICIENT MONKEYS. J. F. Rinehart and (by invitation) L. D. Greenberg, San Francisco, Calif.

*Abstract.* One year ago we submitted the initial observations on the occurrence of arteriosclerotic lesions in the pyridoxine-deficient rhesus monkeys.† At the present time we have studied 10 animals subjected to pyridoxine deficiency. The basic experimental diet is composed of sucrose, 73 per cent; vitamin test casein, 18 per cent; salt mixture, 4 per cent; and corn oil, 2 per cent. When fully supplemented with known vitamins it has supported excellent growth and the animals have remained vigorous and active. In the pyridoxine-deficient animals degenerative and proliferative lesions with fundamental similarities to those seen in human arteriosclerosis have occurred with great regularity. The severity and extent of the lesions have varied with the duration of the experiments which have ranged from 5½ to 20 months.

In the aorta, lesions are first encountered in the lower abdominal segment. Relatively severe lesions are seen in the iliac and femoral vessels. At the same stage such changes have not occurred in the innominate and carotid arteries. A considerable number of animals have developed significant lesions in the coronary arteries and most of the visceral arteries have been involved. The basic lesion seems to involve a loosening of the intima accompanied by proliferation of connective tissue cells. A mucinous ground substance staining red with thionin lies between the separated fibers. The presumption is that the adhesive quality of the ground substance is lost. As in the human disease, eccentric distribution of the sclerotic plaques and a tendency toward localization at areas of bifurcation are

\* Read by title in the absence of the authors. Abstract appears with those of other papers read by title.

† A report of these findings appeared in *The American Journal of Pathology*, 1949, 25, 481-491.

often seen. In a few instances degenerative changes have been seen in the media. In the aorta and iliac vessels, splitting and reduplication of the internal elastic membranes have been observed frequently. The sclerotic lesions are widespread and have been of almost regular occurrence in branches of the renal arteries. Here wavy collagenous fibers in the new-formed thickened intima are coarser than commonly seen in other arteries.

In a considerable series of animals subjected to other deficiencies, lesions of the type described have been absent. Spontaneous arteriosclerosis has not been encountered in the rhesus monkey and this animal has been resistant to the cholesterol lesion. There are a number of considerations other than the direct morphologic evidences presented which suggest that pyridoxine deficiency might be a factor in the pathogenesis of human arteriosclerosis.

### *Discussion*

(Dr. Ernest W. Goodpasture, Nashville, Tenn.) Did you see no particular degenerative change in the elastic tissue of the animals? There was no breaking-up, disintegration, or calcium deposit in the elastic tissue?

(Dr. Rinehart) Yes, the elastic tissue has been interrupted or disintegrated in some of these vessels; the new-formed elastic tissue has often appeared imperfect.

(Dr. William R. Platt, Camden, N.J.) According to Dock the intimal coat of the coronary artery in the male is thicker at birth than in the female. I would like to ask Dr. Rinehart whether he noticed any difference in the degree of coronary arteriosclerosis produced in the pyridoxine-deficient male and female monkeys.

(Dr. Rinehart) Some of the changes I have shown are of a type that are often considered to be those of ageing. However, these changes have not occurred in the control animals or in animals subjected to other deficiencies over a long period of time. Most of our animals have been males.

(Dr. G. Lyman Duff, Montreal, Canada) Have you been able to determine whether there is any lipid deposit in the intima?

(Dr. Rinehart) Fat stains have been done on representative lesions and there is surprisingly little fat seen. Some of the stellate cells contain a few fat granules. These animals are getting a very small amount of fat in the diet; it contains only 2 per cent corn oil.

(Dr. Russell L. Holman, New Orleans, La.) Have you speculated as to the rôle of hyaluronidase? Have you tried vitamin E as a potential preventive of the lesion?

(Dr. Rinehart) The animals have all received vitamin E. I think that is one superior aspect of our experiments; in some experiments of others vitamin E was not included in the diet.

In reply to Dr. Holman's other question, we have speculated as to the rôle of hyaluronidase. There is a mucoïd material in the intima and media which is apparently not normally or properly formed. I think this ground substance is probably closely related to hyaluronate.

**THE LIPID CONTENT OF THE AORTA IN RELATION TO THE NUTRITIONAL STATE AND OTHER CONDITIONS.** Alvin J. Cox, Jr., and (by invitation) Charles D. Miller, San Francisco, Calif.

*Abstract.* Weights of extractable lipid from 250 aortas were compared with standards for individuals of corresponding ages, and the amount of lipid in each case was expressed in terms of the deviation from the standard. This provided objective quantitative measurements which could be compared without respect to age groups. Pronounced increases in aortic lipid were found in the groups of cases of diabetes and of arterial hypertension. The average aortic lipid value was signifi-

cantly lower in individuals who had little stored fat in areolar tissue than in those who were well nourished at the time of death. This confirms similar observations by Wilens. It has not been possible to relate differences in amount of aortic lipid to specific differences in diet.

### *Discussion*

(Dr. Jesse E. Edwards, Rochester, Minn.) Dr. Cox has shown an increase in the lipid content of the aorta in diabetes. His figures tend to show that arterial hypertension has an influence on the deposit of these substances in the aorta. I wonder whether the figures involving patients with hypertension were broken down as to age, since probably the majority of those with hypertension were in the older age groups. Consequently, there might be an age factor which would tend to bring the patients with hypertension into the group of patients with high lipid content.

(Dr. Esmond R. Long, Philadelphia, Pa.) Dr. Cox's comments on the possible practical significance of the lesions he has described remind me of the fact that sometimes starving peoples find some virtue in their deprivation, to make up for what they have gone through. I recall that after World War I some of the German vital statisticians made the point that, although the German people suffered severely in food intake, there was a considerable reduction in the number of deaths from coronary thrombosis. The explanation was offered that, because there was so much less lipid in the diet, they escaped some evils of the type Dr. Cox has just illustrated. I would like to ask Dr. Cox if he knows if the severe malnutrition that has occurred in some parts of the world in recent years has had any effect on the development of atheromatous disease.

(Dr. Cox) In reply to Dr. Edwards' comment, I should emphasize that all of these cases have been compared with standards for the appropriate age so that we feel that the age factor has been eliminated in this way as far as comparison of the cases is concerned. I do not think it is the factor of age which makes the hypertension cases fall into different groups.

With regard to the influence of nutrition in people subjected to starvation, I can only wonder, as Dr. Long has done, whether it could be one virtue of starvation.

**CARDIAC CIRRHOSIS.** James S. McCartney, Minneapolis, Minn.

*Abstract.* Cirrhosis in a person who has had one or more bouts of cardiac decompensation is common, but that the cirrhosis is to be attributed to chronic passive congestion is doubtful. One may find cirrhosis of mild or severe grade which can by no means be correlated with the duration of the clinical evidences of heart failure. If one rejects from consideration those cases with cirrhosis and heart failure plus a history of alcoholism, syphilis, acute catarrhal jaundice or of other possible hepatotoxic agents, the number of instances in which cirrhosis may possibly be attributed to heart failure becomes rather small.

During a 6 years' period the diagnosis of cirrhosis has been made 376 times, or in 2.5 per cent of autopsies. I have examined sections stained for connective tissue from 291 of these cirrhotic livers and have rejected 20 cases because they showed no cirrhosis. In the remaining 271 instances some form of organic heart disease was present in 139, or 50 per cent. From the 271 cases I have selected 64 in which historical or other evidence indicated bouts of heart failure. In 30 instances death was attributed to heart disease, in 16 to cirrhosis, and in the remaining 18 to a variety of causes such as fracture or operation. In 36 instances there was some reason to believe that part of the patients' symptoms might have been due to cirrhosis. The persons with histories of one or more bouts of decompensation of the heart had had such symptoms for periods ranging from 1½ months to 17 years. Two patients had congenital heart disease. One died at the age of 8

years and the other at the age of 28 years. The former had a patchy cirrhosis and chronic passive congestion. The latter had a marked portal cirrhosis, but the signs of passive congestion were not found. In 47 of the 64 I considered the cirrhosis as slight, early, patchy, or subcapsular. Two livers showed hemochromatosis and 2, metastatic carcinoma, and so were rejected. Of the remainder, 4 should be rejected because of chronic alcoholism and 1 because there was a terminal acute yellow atrophy. This leaves, at most, 8 in which chronic passive congestion might conceivably be accused of being the cause of cirrhosis. One had syphilis and so should be excluded. Of the other 7 only one showed cirrhosis of more than moderate degree. Four with a moderate degree of cirrhosis had had periods of decompensation for 17, 5, 2, and 1 years and in 2 the duration of cardiac decompensation is unknown. In none of these livers was there a definite increase of connective tissue in the central portion of the lobule. The only one with severe cirrhosis was a patient with congenital heart disease who died at the age of 28 years.

Conclusions. 1. Cirrhosis of the liver and cardiac decompensation are not infrequently associated. 2. When associated there is little, if any, reason to believe that the cirrhosis is the result of the chronic passive congestion.

### *Discussion*

(Dr. Jesse L. Carr, San Francisco, Calif.) Our figures today are that around 4 per cent of all cirrhotic livers are of this type. I wonder what the actual figures for cirrhosis are in your autopsy series.

(Dr. McCartney) The incidence of cirrhosis in routine autopsies in our series is about 2.5 per cent, which is considerably lower than in many other cities of the country, particularly the coastal cities, where I understand it runs in the neighborhood of 5 per cent. Over the years our figures have averaged 2.5 per cent.

### GLOMERULAR ISCHEMIA IN LOWER NEPHRON NEPHROSIS AND IN THE HEPATORENAL SYNDROME. A. James French, Ann Arbor, Mich.

*Abstract.* The morphologic changes usually described in the renal tubular epithelium in lower nephron nephrosis and in the hepatorenal syndrome cannot be considered sufficient to explain the clinical and laboratory evidences of azotemia. Changes have been found in the glomeruli in cases of oliguria and azotemia of other types which were similar to those designated as glomerulonephrosis in cases of eclampsia by Fahr. These glomerular changes, as associated with eclampsia, had been noted by Löhlein and Leusden and were emphasized by Bell. Trueta has stressed the physiologic findings in renal ischemia.

Twenty cases were selected for detailed study from more than 100 necropsies in which similar glomerular lesions were found to some degree. All cases were eliminated which showed complicating renal diseases, such as glomerulotubular nephritis, arteriosclerotic and arteriolosclerotic nephropathy, diabetic glomerulosclerosis, "wire-loop" lesions in cases of lupus erythematosus, amyloidosis, infarction, and pyelonephritis. The diagnoses in the cases which were utilized included: biliary and atrophic cirrhosis, incompatible blood transfusion reactions, sulfonamide hypersensitivity, acute fibrinopurulent peritonitis, acute yellow atrophy of the liver following arsenical therapy, homologous serum hepatitis, acute hepatitis subsequent to septic abortion, crush syndrome, typhoid fever, carcinoma of the endometrium, myeloid leukemia, and Hodgkin's disease.

The glomerular changes consisted of relative ischemia and thickening of the walls of the capillaries. At least in its early stages, the process appeared to be retrogressive and without increase in connective tissue elements. Even when the thickening of the wall was not sufficient to occlude the capillary lumina, the latter were relatively free from erythrocytes. Proliferation of glomerular epithelium and

increased glomerular cellularity were not prominent findings. The tubular changes in these kidneys were comparable to those described by Bywaters and Beall; Bywaters and Dible; Lucké; Lucké and Mallory; Mallory; Coller, Campbell, and Iob; Darmady; and Ayer. In addition, protein casts, gray with routine hematoxylin and eosin stains, were a common finding in our material and were comparable to the casts in cases of hyperglobulinosis, as noted by Teilum. Tubular lipoidosis was not a conspicuous change.

The retrogressive changes in the walls of the glomerular capillaries were attributed to ischemia. Hyperglobulinosis was a prominent clinical finding in many of the most striking cases in the series. It seemed reasonable to assume that a toxic substance, perhaps a split protein product, might explain the renal changes, including the ischemia, in certain examples of the hepatorenal syndrome and of lower nephron nephrosis.

### *Discussion*

(Dr. Bernard Black-Schaffer, Durham, N.C.) Since Dr. French referred to the work of Trueta, I wonder if he was able to find evidence of any functioning juxta-medullary glomeruli.

(Dr. Lee W. Foster, Chamblee, Ga.) Have you observed this lesion in deeply jaundiced infants showing the morphologic changes of lower nephron nephrosis in the absence of the usual clinical symptomatology?

(Dr. French) In answer to the first question about functioning juxta-medullary glomeruli, we were interested, of course, in the relationship between the outer cortical glomeruli and those in the juxta-medullary region. The changes reported are diffuse and I do not feel that there was a detectable difference between the glomeruli at the juxta-medullary junction and those that were near the capsular surface. I do not feel that we had confirmation of Trueta's work in that regard.

As far as the infants were concerned, we had examples of lower nephron nephrosis, particularly in infants who received sulfonamides. I purposely eliminated them from this report.

### **"LOWER NEPHRON NEPHROSIS" A MISNOMER FOR THE "CRUSH" KIDNEY.**

J. F. A. McManus and (by invitation) Guy L. Rutledge, Jr., Birmingham, Ala.

*Abstract.* The kidneys have been examined by histologic, cytologic, and histochemical methods in a series of fatal cases of trauma. Those dying with the "crush" lesion showed the usual features of relatively normal glomeruli; dilated proximal convoluted tubules with débris in their lumina; pigmented and other casts, especially in the distal convoluted and collecting tubules; tubulo-venous thrombi after 3 days; hyperplasia of the granular cells, with adequate fixation and tissue handling. In addition, it is pointed out that there are no colloid droplets in the epithelium of the proximal convoluted tubules, despite the consistent albuminuria displayed in these cases. The lack of athrocytosis is interpreted to mean a metabolic defect of the proximal convoluted tubules. This abnormality is confirmed by the lack of alkaline phosphatase in the epithelium of the proximal convoluted tubules in groups of nephrons and diffuse decrease in the alkaline phosphatase of all the nephrons. It would seem from these observations, in which the functional and morphologic features of the proximal convoluted tubules are regularly impaired, that lower nephron nephrosis is an inexact, if euphonious, term for a diffuse kidney disease.

**VARIANTS OF CHRONIC GRANULOCYTIC LEUKEMIA.** James Butcher and Edward C. Meek, Jr. (by invitation), and R. Philip Custer, Philadelphia, Pa.

*Abstract.* The manifold expressions of chronic granulocytic leukemia, both clinical and pathologic, have engendered an unnecessarily complex nomenclature to



denote numerous variants of the disease. Some of the terms do not imply any relationship to leukemia and, indeed, certain of the variants are regarded by some authors as being quite different diseases. In an effort to clarify the situation, some of the more variable features of the disease are considered, as indicated in the following tabulation:

<i>Typical</i>	Data	<i>Variant</i>
Adult	AGE INCIDENCE	Congenital or childhood
Present	CLINICAL MANIFESTATIONS	Absent
Increased	GRANULAR LEUKOCYTE COUNT	Normal or decreased
Predominantly neutrophilic	TYPE OF GRANULAR LEUKOCYTE	Eosinophilic or basophilic
Predominantly mature	MATURITY OF GRANULAR LEUKOCYTE	Acute myeloblastic crises
Normal or decreased	ERYTHROCYTE COUNT	Increased
None to moderate numbers	CIRCULATING NUCLEATED RED BLOOD CELLS	Great numbers
Normal or decreased	THROMBOCYTE COUNT	Increased
None to moderate	ASSOCIATED MEGAKARYO- CYTOSIS*	Excessive
Excessive granulo- poiesis	BONE MARROW	Fibrous or bony replacement

We would like to use primarily the generic term, chronic granulocytic leukemia, and subordinate the type as indicated: congenital or childhood, subclinical, sub-leukemic or aleukemic, eosinophilic or basophilic, acute relapsing, erythrocythemic, leukoerythroblastic, thrombocythemic, megakaryocytic, and osteosclerotic or myelofibrotic.

#### *Discussion*

(Dr. Harrison Latta, Wellesley, Mass.) I would like to ask whether the cases in which the marrow first showed a leukemia and later sclerosis or fibrosis had any corresponding changes in the other sites of infiltration at autopsy.

(Dr. Joseph E. Pritchard, Montreal, Canada) Dr. Butcher referred to osteosclerosis; I am not clear whether he means osteosclerosis or myelofibrosis.

(Dr. Butcher) In answer to Dr. Latta's question, one case in which an early biopsy was typical of leukemia, later developing osteosclerosis, showed leukemic metaplasia of lymph nodes. The nodes were not sectioned in 2 other cases from our older files; one was not autopsied, and we did not perform the autopsy in the second. In the latter instance we can only presume that nodes were not significantly enlarged. Because our data were incomplete, we listed this case as questionable.

As to Dr. Pritchard's question on osteosclerosis and myelofibrosis, 2 cases showed extensive increase in bony trabeculae; another, merely displacement of marrow by fibrous tissue without associated proliferation of bone. Osteosclerosis, as we interpret it, implies proliferation of bony trabeculae, and it is usually associated with some increase in fibrous tissue in the remaining marrow spaces. Myelofibrosis denotes simply fibrous replacement of marrow.

#### PATHOLOGY OF YELLOW FEVER IN 1948 OUTBREAK OF SYLVAN FORM IN PANAMA.

Norman W. Elton and (by invitation) Juan Miguel Herrera, Ancon, Canal Zone.

*Abstract.* In November-December, 1948, 5 patients in Santo Tomás Hospital, Panama City, died within 5 to 8 days of the onset of acute febrile disease associated with icterus of a low grade. Post-mortem examinations were made in all

cases, but since the microscopic liver lesion in the first (7435) was consistent with the fulminant form of epidemic hepatitis, only gross examination was made on the next two (7441, 7445). A small amount of liver was taken from the fourth (7468), and a liberal amount of tissue from several organs of the fifth (7509). Review of the liver sections on consultation, January 14, 1949, disclosed the typical lesion of yellow fever in the last two (7468 and 7509). This diagnosis was promptly confirmed by the Army Institute of Pathology.

All of these cases occurred in farmers living east of the Panama Canal between Bayanno River and the Zone boundary on the Pacific side of the Continental Divide, and in each instance hunting or agricultural work had caused the person to be in contact with the edge of the forest for an appreciable period of time during the week preceding the onset of his fatal illness. Although all 5 cases were epidemiologically and clinically consistent with yellow fever, discussion will be confined to the 3 on which liver specimens are available.

7435. Age 25. Peñoncito. Hunting trip on horseback into mountain forest. Died November 12th after 5-day illness. Chills, vomiting, diarrhea, abdominal pain. Non-icteric on admission. Became delirious, with convulsions and areflexia. Slight icterus noted at autopsy. Liver coarsely mottled, reddish on yellow. Duodenum, jejunum, and upper ileum hemorrhagic.

7468. Age 24. Rio Utivé. Weeding rice on edge of forest. Died December 5th after 5-day illness. Malaise, chill, headache, high fever, vomiting. Injected conjunctivae. Slight icterus, but had been taking atabrine. Slight icterus noted at autopsy. Liver diffusely mottled with small ruddy spots on pale yellow background.

7509. Age 16. Pedregal. Working in field at edge of forest in Tapia district. Died December 30th after 5-day illness. Chill, fever, malaise, general aches and pains, followed by epistaxis, vomiting, headache, and sore throat. Non-icteric on admission. Injected conjunctivae. Liver tender. Icterus noted at autopsy. Liver homogeneously yellow on cut surface. Punctiform hemorrhages in jejunum and ileum.

The diagnosis of yellow fever in cases 7468 and 7509 is based upon the typical microscopic liver lesion of the entity. Councilman degeneration of polygonal cells in all lobules was diffuse with the exception of the cells ringing the central veins and those at the periphery, which were in both regions almost intact. No central zones were obliterated. Stains with Sudan III demonstrated the characteristic intense fatty degeneration of the entity.

The liver pattern in 7435 was atypical and consistent with that occasionally observed in fulminant epidemic hepatitis. Curiously, necrosis involved only the peripheral zones in most lobules, although in some it was complete. Councilman degeneration was not distinctive, but fatty degeneration and inflammatory cellular infiltration were marked.

### *Discussion*

(Dr. Ralph D. Lillie, Bethesda, Md.) Were mouse protection tests done on the sera of the surviving suspects? If the adrenal was obtained from the one case in which you had more than liver for examination, was there any cell necrosis seen in the adrenal cortical cells? I have seen that in at least one yellow fever case.

(Dr. Virgil H. Cornell, Washington, D.C.) Have you ruled out Weil's disease in these studies, in view of the past history of yellow fever?

(Dr. Elton) The adrenals were not studied microscopically. The whole thing happened so quickly that few, if any, mouse protection tests were done before the outbreak had ended, and it was only in retrospect that it was suspected some of the recovered cases might have been yellow fever. They will be studied epidemiologically and, if they have not been vaccinated in the meanwhile, by protection tests. It will be determined what they were doing and where they were the week before they became sick. All cases in which the pathologic diagnosis was made checked epidemiologically as the sylvan form of yellow fever.

As far as Weil's disease is concerned, we never considered it because everything was so characteristic of yellow fever.

(Dr. Cornell) That's what Noguchi thought.

(Dr. Elton) Yes, but more water has passed over the dam since that time.

EXPERIMENTAL TOTAL MIDZONAL HEPATIC NECROSIS. B. Black-Schaffer and (by invitation) David S. Johnson, Durham, N.C.

*Abstract.* Rabbits given desiccated thyroid powder (0.73 gm. per diem) until a weight loss of 25 per cent is achieved, react to 0.1 cc. of chloroform per kg., given subcutaneously, with total midzonal necrosis of the entire liver. The central and periportal areas are intact except for loss of glycogen in the periportal zones. Numerous and varied control animals given the same dose of chloroform develop central and focal hepatic necrosis. It appears that under the conditions of the experiment, the midzones of the rabbit liver lobules are metabolically different from the central and periportal zones in a qualitative and/or quantitative sense. It is certain that this unique type of liver necrosis may not be explained solely as a consequence of impaired lobular blood supply (Himsworth).

#### *Discussion*

(Dr. Norman W. Elton, Ancon, C.Z.) I would like to ask if studies of the disturbance in the bilirubin excretion mechanism were performed in the course of these experiments, and whether the rabbits developed clinical jaundice; if so, what type it was, and its course, because the jaundice in yellow fever, for example, is late in manifesting itself clinically. There is no doubt it is there serologically or chemically, if bilirubin tests are performed, but a central zone involvement is the commonest etiologic factor in jaundice. I cannot quite place what the rôle of a midzonal necrosis would be in affecting the van den Bergh reaction by the ring test technic.

(Dr. Ralph D. Lillie, Bethesda, Md.) I have seen in DDT poisoning in experimental animals, on occasion, zonal necrosis quite sharply limited to the mid-zone, I think.

(Dr. Black-Schaffer) In answer to Dr. Elton's question, we have not drawn analogies to human disease since we have not studied the process beyond the point of anatomic observations. None of our animals was permitted to survive more than 24 hours. Whether jaundice would occur after that time we do not know. We have had a batch of animals which we tried to maintain but we found ourselves faced with a very difficult problem; the animals had lost 25 per cent of their body weight, their appetite was severely impaired and the use of vitamin B<sub>12</sub> does not improve it, as far as our experience has gone. I find it difficult, therefore, to answer your question, aside from saying that the conclusions I have drawn are all which I am justified in drawing.

Dr. Lillie's comments are interesting. It is true that there are experiments in which focal midzonal necrosis has been described. Himsworth has attempted to explain this on the basis of intralobular circulation. I think that this is the weakest link of his entire thesis. It is very probable, however, that his thesis may be correct in explaining periportal and central necrosis in many forms of intoxication.

VIRUS HEPATITIS OF CAPTIVE WILD BIRDS. H. L. Ratcliffe and Balduin Lucké, Philadelphia, Pa.

*Abstract.* During the spring of 1946, a hitherto unrecognized form of hepatitis developed among birds at the Philadelphia Zoological Garden. This disease has continued to the present time and has caused the death of over 150 birds which

were representative of 20 Orders and of approximately 40 Families. Usually birds have died during the acute phases of the disease without showing obvious signs of illness. Less frequently the fatal hepatitis occurred in subacute or chronic form, manifesting itself by reduced activity, loss of appetite, and loss of weight. In the acute form the liver was enlarged, sometimes four-fold. Other viscera exhibited no significant gross changes. In the subacute stage the liver also was usually larger than normal but more or less distorted by the formation of coarse nodules in some areas and shrinkage in other areas. With the chronicity of the disease, distortion of the liver became more pronounced. Subacute and chronic hepatitis was accompanied by edema of the subcutaneous tissues and the muscles, accumulation of fluid in serous cavities, pulmonary edema, and dilatation of the heart.

Microscopic sections of these livers suggests that the basic lesion of avian hepatitis is more or less extensive focal necrosis of liver cells throughout the organ. At times this may be so severe that almost every cell appears necrotic and death occurs before an appreciable reaction develops, but usually necrosis has been limited to many smaller or larger foci, in and about which a few leukocytes and many macrophages accumulate. In such livers macrophages also have been increased throughout the capillary bed of the organ. Intact liver cells contained globules of bile pigment. In examples of the subacute disease, necrotic foci were less conspicuous, but regions were found in which the liver cells had disappeared, leaving collapsed reticulum, macrophages, and bile ducts. In other areas the parenchyma was either intact or had formed regenerating masses of large cells. In chronic hepatitis the livers were composed largely of regenerating nodules which lacked lobular arrangement. These were separated by bands of compressed tissue in which bile ducts and macrophages were numerous. Thus, the morbid anatomy and histopathology of this disease simulates, in many respects, the pattern of epidemic hepatitis in man.

Bacteriologic studies of these livers have been negative, and nutritional factors have been excluded. On experimental grounds avian hepatitis has been attributed, tentatively, to a virus. The source of this disease has not been established, but epidemiologic surveys have revealed that spread within the Zoological Garden may be attributed to direct contact, or to contaminated food, and to the activities of keepers in cleaning cages.

#### *Discussion*

(Dr. Ernest W. Goodpasture, Nashville, Tenn.) Did you find any histologic evidence of acute destruction of entire lobules in the liver?

(Dr. Lucké) No, we did not. There was in none of the birds a wiping-out of a third or a half of the liver; the lesions were more scattered.

(Dr. Goodpasture) Is the disease easily transmissible from animal to animal?

(Dr. Lucké) By means of cell-free filtrates we have been able to transmit the disease.

(Dr. Goodpasture) What is the incubation period?

(Dr. Lucké) This apparently depends on dosage and varies with the species.

(Dr. Russell L. Holman, New Orleans, La.) Have any of the attendants had infectious hepatitis, or do you think the birds could be the reservoir for infectious jaundice?

(Dr. Lucké) It is impossible for us to be certain whether any of the attendants have had epidemic hepatitis. There are certainly a number who might have had it. We believe that human hepatitis can be transmitted to birds.

ISOLATION OF BRUCELLA FROM APPARENTLY HEALTHY INDIVIDUALS. Leon V. McVay and Frances Guthrie (by invitation), I. D. Michelson, and Douglas H. Sprunt, Memphis, Tenn.

*Abstract.* In a paper read before this society in 1942 by W. D. Forbus and his associates, it was reported that brucella organisms had been isolated from several cases of Hodgkin's disease. These findings have been confirmed in our laboratory. It was thought that an explanation of this might be that in an endemic area brucella may localize in chronically damaged tissue. Therefore, chronically infected tissue was cultured for these bacteria.

Two hundred and fifty surgical and autopsy specimens were studied bacteriologically for the presence of brucella organisms. In 4 cases, *Brucella melitensis* was isolated, and in 2 others, *Br. abortus* was found. These specimens were studied microscopically and a vasculitis was observed in 4 of them. Eighty-two prostates were cultured. *Br. abortus* was isolated in 2 instances and *Br. melitensis* in an additional 2 cases. Ninety-three fallopian tubes were cultured. From 2 of these *Br. melitensis* was isolated. Seventy-five autopsy specimens consisting of liver, spleen, lymph nodes and fibrosed areas of lung were similarly studied without success. The cultures of the surgical specimens were made by obtaining from the surgeon, in a sterile container, fallopian tubes or portions of prostate which had been removed through the urethra. Autopsy specimens were acquired as aseptically as possible, and the surfaces were treated with a searing iron. All specimens were immediately macerated, extracted, and the tissue extract incubated in bacto-tryptose broth at 37.5° C. in 10 per cent carbon dioxide for 10 days. After 10 days the broth specimens were inoculated on bacto-tryptose agar plates, and these were similarly incubated for 10 days. Unless positive specimens were obtained sooner, this procedure was repeated twice before discarding the specimens as negative. Identification of the organisms was made by (1) the character of the colony on tryptose agar, (2) the form and staining reactions of the individual organisms, (3) sugar reactions, and (4) by agglutination with specific antiserum.

The specimens yielding brucella on culture were studied microscopically. One prostatic specimen could not be evaluated because of widespread malignant involvement. The remaining 3 showed evidence of a non-specific, chronic inflammatory process of moderate degree. There was vasculitis in 2 of the prostatic specimens and, to a lesser degree, in the fallopian tubes. This involved the small vessels: arterioles, venules, and capillaries. There was thickening of the endothelium with eosinophilic infiltration. Microscopic study of the bacteriologically negative specimens failed to reveal this lesion. Perivascular cuffing by lymphocytes, eosinophils, macrophages, and plasma cells was noted. Hyalinization and vacuolization of the walls of many small vessels was observed.

Five of the patients whose tissue yielded brucella have been carefully studied. All gave a clinical history compatible with brucellosis. All of them had lived on farms and had drunk non-pasteurized milk. In one case there was known exposure to Bang's disease. In 3 cases, 3 intradermal brucella antigens applied simultaneously gave strongly positive reactions. In the fourth case, 2 antigens gave positive reactions, while in the fifth case only 1 antigen showed a positive intradermal test. No specific agglutinins could be demonstrated in the serum of any of the patients. In no case was there a history of a recent episode suggestive of active brucellosis.

#### Discussion

(Dr. Wiley D. Forbus, Durham, N.C.) The experience of this group of workers is to me not a surprising one. If one makes a large survey, and, of course, this is a considerable series, I think almost any population, certainly any population that

is semi-rural, will yield results of a similar sort. If such a survey is made with repeated cultures, then the chances of increasing the number of positives are, of course, greatly increased. In our own studies of the occurrence of brucella in connection with the Hodgkin's cases we had this experience: We might go for a period of a month or 2 months with repeated cultures and get nothing, and then after about the 30th culture, out would come the organism. So this organism does lie around, if I may be permitted to use that expression, in human tissues, but I am not at all sure that it lies around always in chronically infected tissues. Certainly, our experimental experience would indicate that the presence of no form of irritation of the tissues, infection, or what have you, is required for the localization of the organism. In our studies on the hog, the monkey, and a variety of other animals, we have inoculated brucella in great numbers without producing any form of disease. We have studied these animals at autopsy often well over 300 days, and we have been able to isolate the organism from lymph nodes showing no reaction which might be interpreted as chronic inflammation. I say again that the results of the studies of Dr. McVay and his associates are not surprising, but those which might have been predicted, especially since the study was made on a population which undoubtedly is subject to infection by this organism.

(Dr. W. T. S. Thorp, Bethesda, Md.) Does Dr. McVay consider the intradermal test as evidence of brucellosis?

(Dr. McVay) Not by itself.

(Dr. Thorp) Did you make agglutination tests on individuals who were negative to culture?

(Dr. Leo Lowbeer, Tulsa, Okla.) The interesting phenomenon of a subclinical infection caused by a notoriously pathogenic microorganism is well known from animals like goats and non-pregnant cows, both of which may carry enormous numbers of brucella in their milk-ducts without clinical disease. There are also some reports on subclinical infection in men, particularly those exposed to brucella, like veterinarians or packing house employees. These reports are based on positive blood cultures and agglutination in significant titers in asymptomatic individuals. The existence of a subclinical brucella infection is apparently related to the capacity of brucella to survive as an intracellular organism within mesodermal macrophages and in ectodermal cells of such organs as bone marrow, spleen, lymph nodes, tonsils and gallbladder wall, as shown by Theobald Smith, Goodpasture and Anderson, K. F. Meyer, Castaneda, Boak and Carpenter, Buddingh and Womack. This intracellular parasitism is associated with recurrent clinical disease, if the parasite overcomes the defense mechanism of the host. If, on the other hand, the surviving parasite is held in check by the defense mechanisms, there will be no clinical disease: subclinical infection. The occurrence of clinical disease then depends more on the balance between the rate of growth and virulence of the surviving intracellular parasite, and the humoral and cellular defense mechanisms of the host, than on the fact that the parasite survives. Since nothing is known on agglutination and skin allergy in the cases presented, I should like to ask the authors whether the virulence of the cultured brucella was tested by inoculation of guinea-pigs.

(Dr. McVay) We did not check the virulence on the guinea-pig. We did not check the other patients for intradermal reactions; however, in an attempt to compensate for that we selected 100 random patients and obtained 39 positive reactions, of which 5 were strong. In doing them we found 2 patients with active brucellosis who had fever and chills. Certainly the skin test by itself is not indicative of the diagnosis.

CELLULAR CHANGES IN SYSTEMIC LUPUS ERYTHEMATOSUS. Paul Klemperer and (by invitation) Boris Gueft and Stanley L. Lee, New York, N.Y.

*Abstract.* "Clumps and packets of hematoxylin-stained bodies" in endocardial vegetations of acute systemic lupus erythematosus were described by Gross. Subsequently Ginzler and Fox reported the finding of similar large "hematoxylin-staining bodies" in necrotic lymph nodes of a case of this disease. Both Gross and Ginzler and Fox recognized the probable origin of these bodies from chromatin material. These observations were confirmed in a large series of autopsied cases. Moreover, similar cellular changes and their evolution were observed in widely disseminated sites, such as serosal membranes, spleen, synovia, mediastinal connective tissue, vagina, skin, and glomeruli, always limited to cells of mesenchymal origin.

Histochemical analysis with Feulgen reaction, methyl green, methyl green-pyronin, ribonuclease, and ultraviolet absorption spectrophotometry reveal that the basic nuclear structural alteration is due to partial depolymerization of desoxyribosenucleic acid. (These studies were carried out with the assistance of Drs. Polister and Leuchtenberger of Columbia University.) These observations suggest that one factor in the morbid process of acute lupus erythematosus is a disturbance of nucleic acid metabolism manifesting itself in depolymerization of the desoxyribosenucleic acid of the cells of the mesenchyme.

#### *Discussion*

(Dr. Robert J. Parsons, Oakland, Calif.) I would like to ask what fixative was used, and if these bodies were seen after all fixatives.

(Dr. Klemperer) The hematoxylin-stained bodies can be seen in tissues fixed in any fixative. For histochemical analysis fixation in Carnoy fluid or acetic acid alcohol is preferable. In the last 3 cases material fixed in this way was available for our studies.

POST-PARTUM ENDOMETRIAL HYPERPLASIA IN DIABETIC PATIENTS TREATED WITH STILBESTROL AND PROLUTON. William A. Meissner and (by invitation) Sheldon C. Sommers, Boston, Mass.

*Abstract.* Pregnant patients with diabetes mellitus are prone to abort, to have premature deliveries, and to have stillborn fetuses. These complications occur in spite of the fact that insulin requirements are adequately met and the blood sugar is kept under control. The occurrence of such difficulties during pregnancy has been assumed to be due to the deficiency of ovarian hormones which some diabetic females may show. On the basis of this assumption, a large number of pregnant diabetic patients have been carried through pregnancy very successfully with the use of large dosages of stilbestrol and proluton during gestation. In a consecutive series of 50 cases treated with such therapy, post-partum uterine bleeding due clinically to hyperplasia of the endometrium occurred up to 1 year following delivery. The bleeding was often severe, requiring transfusion, and in each instance a dilatation and curettage was performed. The endometrium presented no noteworthy gross features other than its abundance. It was yellow, hemorrhagic, and friable. Microscopically, the endometrium uniformly showed marked cystic and adenomatous proliferation of the glandular epithelium with frequent stratification. The individual epithelial cells showed pleomorphism. There was never conclusive evidence of secretory activity. Mitotic figures were not found in every specimen, but in some there were many. The stroma was unusually active and contained thrombosed sinusoids and eosinophilic leukocytes.

The exact nature and cause of the hyperplasia are unknown, although microscopically it suggests a response to excessive estrogenic stimulation. Subsequent cases

treated with a different brand of stilbestrol (possibly a weaker dose) have failed in most instances to show the same complication. It is possible, but unproved, that the condition is due in part to the diabetic state since the hyperplasia in this series was much more pronounced than that found in a control series of post-partum endometrial hyperplasia in non-diabetic females; moreover, the hyperplasia was more pronounced than that reported in non-diabetic women receiving high dosages of stilbestrol during pregnancy. The hyperplasia was not considered precancerous.

### *Discussion*

(Dr. Joseph E. Pritchard, Montreal, Canada) I should like to ask Dr. Meissner if he noted any vascular changes in the endometrium. I should also like to suggest that this condition might be termed heteroplasia rather than hyperplasia to differentiate it from the more balanced overgrowth of the endometrium; they are all lumped together as hyperplasias.

(Dr. Jesse L. Carr, San Francisco, Calif.) In the cases in which there was infant mortality, did you observe any pathologic changes in the ovaries of the dead infants?

(Dr. Meissner) Dr. Pritchard's suggestion of heteroplasia might be good because this condition is different from the usual hyperplasia, and it does seem to be more heterogeneous than those usually seen.

I am sorry we have no figures on the condition of the ovaries in the dead infants. If a woman had a miscarriage she was not hospitalized in our hospital, so the autopsy on the infant would have been performed in another laboratory.

(Dr. Pritchard) How about the blood vessels?

(Dr. Meissner) They showed no changes other than those usually seen in endometrial hyperplasia. There were no changes which could be attributed specifically to the diabetic state.

## SYMPOSIUM\* ON PATHOLOGY IN FORENSIC MEDICINE

**PATHOLOGY IN FORENSIC MEDICINE.\*** Alan R. Moritz, Boston, Mass.

By definition forensic medicine encompasses all those medical facts and procedures that are required to enforce the law and to administer justice. Since there is no part of any of the special areas between anatomy and zoology that is not occasionally called upon to serve the needs of justice, the potential scientific content of forensic medicine is exceedingly broad. Actually it is not as diffuse as might be inferred from the definition. The principal needs of the law for special medical knowledge fall into two main categories, pathology and psychiatry. This discussion concerns the place of pathology in forensic medicine.

There occur approximately 1.5 million deaths per year in the United States. If the causes of death in those areas from which vital statistics are reasonably reliable are representative of the country at large, it may be estimated that about 300,000 deaths per year result from violence of one kind or another, or occur unexpectedly from obscure causes. The existence in most jurisdictions of laws which stipulate that the cause and manner of such deaths shall be investigated by a public official implies that the acquisition of such information is essential to public welfare. Why is this the case?

In this great pool are all of the homicides, all of the suicides, all of the fatal accidents, and a wide variety of unrecognized rapidly fatal diseases. For the majority there is no reliable history. In many the terminal seizure and death was unwitnessed. Obviously there should be an official agency for obtaining the medi-

\* By invitation of the Council.



cal facts necessary to prevent murder going unsuspected, to prevent the prosecution of innocent persons for murder because of misleading circumstantial evidence, and to prevent non-recognition of controllable hazards to public health.

As you know, the office of county coroner is responsible for the investigation of such deaths in most jurisdictions. Because in most communities this office is under the control of a person whose qualifications are political rather than professional and whose resources for and interest in scientific work are insignificant, its medical fact-finding ability is characteristically negligible.

It is probably a fact that in the average American community the only murders that are recognized as such are those that were witnessed or those in which the victim shows unmistakable external evidence of assault. It would be interesting to know what the actual homicide rate in the United States may be.

It is also a fact that in the average American community the presence of pathologic changes that simulate violence in or on the body of a person whose death occurred in suspicious circumstances is likely to result in the prosecution of whomsoever may have had motive and opportunity to kill. I have known of many prosecutions for murder in which the medical evidence pertaining to the cause and manner of death was totally unreliable.

It is, furthermore, a fact that in the average American community there is no mechanism for compelling the performance of a competent autopsy in the interests of public health. In a community such as Massachusetts, in which the interests of public health are sufficient justification for the performance of a compulsory autopsy, many otherwise unsuspected or unproved hazards to life are disclosed by medicolegal autopsies.

What is the responsibility of American pathology in relation to the investigation of these 300,000 so-called medicolegal deaths? At the present moment there are relatively few communities in the United States in which qualified pathologists are utilized for this important public service.

There should be in every community an official organization which would provide, first, for the screening of all medicolegal deaths, and second, for the acquisition of complete and reliable evidence in those instances in which such information is needed in the public interest. To provide a screening mechanism there should be in each community one or more official physicians to whom all deaths by violence or deaths due to obscure causes are reported for preliminary investigation. If the information derived from a preliminary inquiry and view of the body of the dead person indicates that there is no reason to believe that public welfare is jeopardized, the death may be certified without further investigation.

There should also exist in every city and state a facility for conducting complete and competent official post-mortem investigations whenever the need for such information is disclosed by the preliminary inquiry. Experience in Massachusetts where such a mechanism exists indicates that autopsy is required in the interest of public safety in approximately one of every five of the so-called medicolegal deaths.

Unfortunately, too many pathologists are unwilling or unable to assume responsibility for certain objectives of a medicolegal autopsy that are peculiar to the fact that it is performed for medicolegal purposes. Too often the pathologist confines his investigation to what he construes to be the normal scientific objectives of an autopsy and ignores certain evidence that is fully as important from a medicolegal standpoint as any of the conventional medical facts disclosed.

What are some of the special characteristics of forensic pathology?

One deals with problems in identification. In approximately 5 per cent of the medicolegal autopsies conducted by the Department of Legal Medicine of Har-

vard Medical School the first question to be answered relates to the identity of the dead person. This is particularly true in the case of burned, mutilated, or decomposed bodies. Sometimes the problem is even more basic and concerns determination of whether the fragmentary remains are from man or animal? The pathologist who accepts the responsibility for medicolegal autopsies must be prepared either to make such determinations himself or to see to it that the material in question reaches the hands of a person qualified to deal with it.

The aggregate of many little things, no one of which is necessarily significant by itself, is often of great importance in the establishment of identification. The fragmentary remains of a skeleton were recently found partially buried in the humus of a pine forest. Examination of the bones by an anatomist and a roentgenologist established that the remains were those of a young women having a stature of approximately 52 inches. Animals and flood water presumably were responsible for the fact that many of the bones were missing. The only vestiges of the head were one incisor tooth and a mat of brown hair. A dental expert who examined the tooth recognized a highly distinctive dental deformity. The sex, age, stature, and hair were consistent with those of several missing girls but the recognition of the peculiar dental deformity known to have been possessed by one of them established the individual identity of the remains with reasonable certainty. The finding beside the bones of a stoppered bottle which contained traces of barbiturate together with information from relatives that the missing girl had been exceedingly despondent justified the presumption of suicide.

In approximately 5 per cent of medicolegal autopsies the establishment of the probable time of death is of critical importance. The accuracy desired may be in terms of hours, days, weeks, or years according to the circumstances of the case. If John Doe, whose fatal injuries appear to be compatible with suicide, died yesterday, the presumption of suicide may stand. If, however, the medical evidence indicates that death occurred 2 days ago, the fact that neighbors heard someone moving around in the dead man's room yesterday may justify a presumption of homicide. Thus, a knowledge of the rate and sequence of the changes that occur in the human body after death is an important part of the armamentarium of the forensic pathologist. Most of all he should be aware of the limitations of such evidence pertaining to the time of death. In the case of the skeleton found in the woods, the police investigation was greatly facilitated by information supplied by the pathologist in respect to the time of death. It was reported that the skeleton had lain on the ground where it was found for at least 7 years, and probably longer. This information was provided with the aid of a botanist who examined the roots of trees that had grown through the fragmentary remains of the victim's clothing. Birch roots 5 years old were identified and since several years are required for pine needles to decompose sufficiently to support the growth of a deciduous tree, 7 years was estimated as the minimal duration of the post-mortem interval. Actually the body had lain in the forest for 12 years.

Obviously, the determination of the cause of death with the greatest possible accuracy is desirable in all medicolegal autopsies. Too often a medicolegal autopsy is terminated with the finding of what seems to be an adequate explanation of death and no real effort is made to exclude other possibilities. Many elderly persons have degenerative diseases which, in the absence of evidence to the contrary, might be regarded as probable causes of death. A good illustration of the danger of forming a premature conclusion is provided by the case of an elderly woman who was found dead in bed. An autopsy disclosed that she had pulmonary tuberculosis, a cancer of the cervix, and chronic pyelonephritis. Further investigation established that her death was actually due to arsenic poisoning. Arsenic

was looked for not because there was anything in the history to indicate such a probability but because of unusual hyperemia of the gastric mucosa and the finding of a small amount of white particulate matter in the gastric contents.

Another occasional cause of a pathologist's failure in the investigation of a medicolegal death is to assume that death was caused by trauma because of the presence of external injuries on the body. Recently a woman with a badly bruised face was found dead beside a car in the parking lot of a country tavern. She and her male companion had spent the evening in the tavern and were drunk and quarrelsome. About eleven o'clock the woman left and 30 minutes later he, too, left. Sometime thereafter another guest going to his car saw the woman on the ground and her companion bending over her. Together they brought her into the tavern where a doctor was called who pronounced her dead. She had a bruised face and scalp and autopsy disclosed an intracranial hemorrhage which was at first considered to be traumatic. The suspect said that he had found the woman on the ground when he went outside to look for her. His story was not believed at first. If death had been reported as due to head injury the circumstances were such as to have led to suspicion, if not prosecution, of the dead woman's companion for fatal assault. Actually, a complete autopsy disclosed that death resulted from the rupture of an exceedingly small congenital aneurysm of the circle of Willis.

In the performance of autopsies for medical rather than medicolegal purposes the pathologist is rarely concerned with the acquisition of evidence bearing on the manner or circumstances in which injuries were received. In a medicolegal investigation such evidence may be of paramount importance. Thus the finding of a blood-alcohol level of 0.30 per cent in the pedestrian victim of an unwitnessed fatal automobile accident may confirm the driver's statement that the dead man stepped off the curb directly in front of the car. A pathologist should always be alert to the possibility of illuminating the circumstances that attended the receipt of fatal injuries.

A few months ago a shooting occurred in which the two men involved were approximately 6 feet apart at the time the fatal shot was fired. A 45 caliber bullet passed through the victim's chest from front to back and was immediately fatal. The other man admitted the shooting but stated that it was meant to be a joke and that the gun was not pointed at the dead man when he pulled the trigger. His story was confirmed by the fact that the appearance of the entrance wound in the front of the dead man's chest indicated that the bullet was already badly deformed and exceedingly hot when it first struck him. The finding that the bullet was already both hot and deformed indicated that it had struck some resistant object before striking the dead man's chest. Actually it had first struck a steel shelf and had ricocheted in such a direction as to cause the fatal injury. If this had not been recognized, the death would have led to criminal prosecution.

There are a variety of reasons why pathology participates ineffectively in the practice of forensic medicine in America. One of the most important of these is that in most places forensic pathology is not included in the training of pathologists. A pathologist is not likely to be competent to meet the problems of forensic medicine unless he has had training or experience in dealing with them. We are not going to acquire experience or be able to give adequate training unless we acquire local responsibility for the practice of forensic pathology. If the majority of members of this Association persist in their present relatively passive attitude toward pathology in forensic medicine, the situation will remain unimproved. Those of us who feel keenly the need for improvement are enormously encouraged by the interest evidenced by the allocation of this symposium to a consideration of pathology in forensic medicine.

A STUDY OF THE ELECTRIC RESISTANCE OFFERED BY ANIMAL AND HUMAN TISSUE. R. S. Fisher (by invitation) and Alan R. Moritz, Boston, Mass.

*Abstract.* Studies have been conducted using direct current and silver-silver chloride non-polarizing electrodes with actual skin contact through saline media in glass tubes. The following conclusions are reached:

1. In dealing with tissues, resistance may be expressed in terms of ohms, only for specified electrode areas and lengths of pathway.
2. The skin offers relatively high resistance (700 to 15,000 ohms/3 cm. diameter circular electrode with double thickness skin) to low voltages (6 to 10 volts), but at higher voltages (above 50 to 70 volts) skin resistance becomes very low.
3. Contrary to published reports, internal tissues, especially fat, offer significantly large resistance to the passage of direct current.
4. With intact extremities in humans and trans-thigh sites in pigs, the effective resistance increases as the area of the electrodes is decreased, but the increase is disproportionate to (is less than) the decrease in area. This is explainable by an internal (ellipsoidal) path of larger diameter than the electrode area.
5. Using sodium chloride solutions in the electrodes, there is an apparent asymmetric localization of resistance with that under the cathode being larger than that under the anode. With potassium chloride solutions the anode appears to be the site of higher resistance.
6. Thermal effects (heating) observed under the electrodes appear, in preliminary experiments, to be of sufficient magnitude to explain the injuries incident to the passage of the current.

THE MEDICOLEGAL ASPECTS OF RADIATION INJURY. Shields Warren, Boston, Mass.

*Abstract.* The medicolegal aspects of radiation injury involve the following factors, among others:

1. The pathology of radiation injury.
2. Means of prevention.
3. Responsibility for injuries produced.

As evidence of local radiation injury the triad of (a) wide variation in cellular alterations; (b) increase in, and hyalinization of, connective tissue; and (c) thickening of blood vessel walls or occlusion of blood vessels with or without telangiectasia, is helpful. With atrophic changes in hematopoietic tissue or osteogenic sarcoma in an adult, radiation injury, either external or internal, should be considered. It is the responsibility of each individual handling apparatus or material giving off ionizing radiation to see that he himself, his staff, his patients, and those working nearby are adequately protected.

BLUNT FORCE INJURIES OF THE HEART. Milton Helpert, New York, N.Y.

*Abstract.* Traumatic injuries of the heart from non-penetrating blunt force result only when a severe grade of violence is applied directly to the chest wall. The force may be generalized or localized. In most cases cardiac injuries are associated with other severe chest and bodily injuries and are immediately or rapidly fatal. Generalized blunt force, severe enough to crush or compress the chest and produce cardiac and other intrathoracic injury, is encountered most often in vehicle accidents and in falls from a height. A crushing downward force applied anteriorly may tear the heart more or less completely from its superior and posterior attachments and also tear the pericardial sac. In young persons with a resilient thorax, this may occur without fracture of the ribs or sternum. With less violence, incomplete internal transverse lacerations of one or both auricles above the auriculoventricular valve rings, and of the attachments of the venae cavae and pul-

monic veins may occur; if the victim survives long enough, mural thrombi may develop over the site of injury in the auricle.

Crushing of the chest may produce punctures and lacerations of the pericardium and heart by sharp ends of broken ribs or sternum. There may be a variety of cardiac injuries including tears of the valve cusps and leaflets and of the papillary muscles. Contusions of the auricles or ventricles and injuries of the coronary arteries are rarely encountered and never as isolated injuries. Incomplete internal laceration of the right coronary artery just beyond the ostium was one of multiple severe cardiac injuries in 2 cases. Injuries of the left coronary artery have not been encountered. The anterior position of the right coronary artery makes it more vulnerable than the left. Traumatic injury of a calcified coronary artery and traumatic coronary thrombosis have not been encountered in a large series of blunt force cardiac injuries observed at autopsy.

Sudden violent compression of the chest may produce one or more bursting ruptures of the heart, involving the ventricles, auricles, or septa. The pericardium may not be torn and in young persons the bony thorax may escape fracture. Death ensues rapidly from intrapericardial hemorrhage. Bursting ruptures are the most frequent cardiac injury resulting from falls from a height. Rarely, severe compression of the chest may cause a long bursting laceration of the pericardium and relatively slight contusion of the right auricle or ventricle; in such cases the victim, even with a markedly hypertrophied heart and severe coronary sclerosis, may survive many days and death result only in part from the cardiac injuries.

A severe localized blunt force like that from the impact of a small, hard, fast-moving object, striking the chest over the sternum or just lateral to it on either side, may contuse and rupture the thin anterior wall of the right ventricle without damaging the pericardial sac. The right ventricle is especially vulnerable to violence of this type because of its anterior position. The force is usually severe enough to fracture the costal cartilages or the sternum beneath the site of impact. The ruptures are usually small and, if complete, result in rapid death from hemopericardium. In some cases, rupture of the contused area is delayed several hours or days. In rare cases a contusion may heal with or without formation of a small aneurysm and mural thrombus. The great majority of isolated contusions of the heart occur in the anterior wall of the right ventricle as the result of such localized blunt force impacts.

Traumatic injury of the heart by the mechanism of contrecoup with the force transmitted obliquely upward from the buttocks does not occur and does not merit serious consideration. Bursting ruptures of the heart do not result from indirect violence which compresses the abdomen.

### *Discussion*

(Dr. William R. Platt, Camden, N.J.) I should like to ask Dr. Helpern how he feels about the relationship of trauma to the sternum in young people and the subsequent development of anginal attacks leading to coronary disease and death in a 6-months' period.

(Dr. John J. Clemmer, Albany, N.Y.) Has Dr. Helpern seen pericarditis following injuries of the chest?

(Dr. Victor Levine, Chicago, Ill.) I should like to ask if a less severe injury to the chest might cause damage and laceration of the coronary artery if the artery is very sclerotic.

(Dr. Helpern) The answer to the first question is difficult in the absence of the post-mortem evidence. We have found severe coronary sclerosis not infrequently as a cause of sudden natural death in young adults in whom the disease was entirely asymptomatic and therefore unrecognized and unsuspected during life. It is true that blunt force injury to the chest might precipitate symp-

toms in a person with pre-existing, but previously asymptomatic, coronary disease but it does not cause the disease. It is a mistake to assume that because there have been no prior symptoms there has been no pre-existing disease. Most of the cases we have seen in which symptoms developed and progressed fatally after injury revealed evidence of severe pre-existing coronary arteriosclerosis.

With regard to pericarditis following an injury to the chest, I do not recall any case in which pericarditis developed as a complication of a non-penetrating blunt force injury, although it is conceivable that it might develop as an indirect complication of infection extending from the lung or pleural cavity, or as a manifestation of sepsis complicating infection elsewhere in the body.

In reply to the third question as to a less severe injury to the chest causing damage and laceration of a very sclerotic coronary artery, I should like to emphasize that the coronary arteries whether normal or sclerotic are rarely injured even by the most severe blunt force; therefore lesser degrees of force would also have no direct effect. In the 2 cases which I demonstrated the coronary artery injury was only one of many other cardiac and thoracic injuries. It was not an isolated lesion. I have never seen the left coronary artery similarly injured.

#### SUBDURAL HEMORRHAGES FOLLOWING RUPTURES OF ARTERIES AND VEINS ON SURFACE OF CEREBRUM. Benjamin Morgan Vance, New York, N.Y.

*Abstract.* The findings were reviewed in deaths from subdural hemorrhage due to a rupture of a blood vessel on the surface of the cerebral hemisphere, as encountered among the autopsies in the Office of the Chief Medical Examiner in New York City. Such deaths are important forensically, inasmuch as some of the subjects have been exposed to criminal or accidental violence, and, in cases of this type, it is essential that the lesions causing death should be accurately determined and their connection with trauma properly appraised. Microscopic examinations were made in 10 cases and in 6 the ruptured blood vessels were demonstrated satisfactorily, 3 being arteries and 3 veins. The mechanism of the rupture of blood vessels is discussed.

#### *Discussion*

(Dr. Alfred Angrist, Jamaica, N.Y.) I wonder whether Dr. Vance noticed herniation of the uncinate gyri in these cases of subdural hemorrhage. The reason I ask that question is that therein may be the mechanism for the pontine hemorrhages that are found in association with them. I think such pontine hemorrhages can be produced experimentally by subdural injection of fluid foreign bodies of different sorts. In the group of cases where arterial rupture occurred, and I realize there are very few, is it Dr. Vance's impression that the clinical picture is a more rapid one in contrast to the slower venous bleeding into the subdural space?

(Dr. Vance) To answer Dr. Angrist's last question first, it seemed to me that fatal subdural hemorrhages from ruptured arteries developed in about the same length of time as subdural hemorrhages from ruptured veins. However, as I have investigated only 6 cases in which the ruptured blood vessels were identified as arteries or veins, I cannot speak with authority on this point. As for the uncinate gyri, I do not recall having seen them unduly prominent in cases in which discrete pontile hemorrhages were present.

#### THYMIC HYPERTROPHY AND AURICULAR COMPRESSION. Jesse L. Carr, San Francisco, Calif.

*Abstract.* In a post-mortem study of approximately 1500 autopsies on babies under 1 year old, dying suddenly, and worked up at the San Francisco Coroner's Office, numerous causes of death have been found, and among them there have been included lymphatism, status thymico-lymphaticus, status thymico-asthmaticus.

cus, and combinations of these three. In a certain percentage of children dying with a large thymus and with the mechanism of death otherwise unexplained it has been reasonably possible to ascribe death to compression by a large thymus of the auricles, particularly on the right side. In a relative sense, the auricles in these children are appreciably smaller than in normal babies of the same age, and at autopsy the auricles are found to be empty. There are also certain other upsets in the dynamics of circulation in these children, which we believe to be of importance in explaining their sudden demise. This material, together with microscopic studies of the lungs, heart, and lymphatic system, is included.

### Discussion

(Dr. Jesse E. Edwards, Rochester, Minn.) I should like to point out that in certain newborn infants with serious respiratory difficulties, dissection of the thymus, even a large thymus, from the mediastinal structures will uncover a malformation of the aortic arch which compresses the trachea and which is the basis for the respiratory distress. That this point is stressed is justified by the fact that for some of the vascular malformations to which I refer there are available surgical technics which may be lifesaving, if employed.

(Dr. Jacob Werne, New York, N.Y.) In 1942 it was shown before this Society that a series of normal infants killed instantly by violence showed as large, or larger, thymic glands than any which have been described in so-called status thymico-lymphaticus. If Dr. Carr will autopsy infants dying of violence in his jurisdiction, he will confirm the fact that a normal infant's thymus is large. With respect to the thymic hemorrhages, I will have more to say during our presentation of histopathologic study of actual and alleged asphyxial deaths. The thymic petechiae seen in so-called status thymico-lymphaticus are referable to vascular injury resulting from fulminating infection, usually of respiratory origin.

(Dr. Frank Dutra, Cincinnati, Ohio) One fact which leaves me totally unconvinced on this matter is that these children die suddenly without any evidences of cardiac decompensation. In the ordinary situation where there is a tumor growing against and compressing the right auricle there is the development of marked peripheral evidences of chronic venous stasis. There is no evidence in any of the cases presented that there has been any such stasis, in spite of this alleged severe pressure on the right auricle.

Anyone who does autopsies knows that it is common to see hemorrhages scattered throughout the thymus as a terminal event, and this is absolutely insignificant as a factor in predisposing death. Small hemorrhages are commonly found in the thymus when death was preceded by anoxemia or infectious disease.

I should like to say one thing more. In order to demonstrate abnormal anatomic relationships, the organs must be examined before those relationships are destroyed. Certain conclusions about the possibility of one organ compressing an adjacent one would be totally invalid if the observations on which they were based were not made while the organs were *in situ*. Even removal of the viscera *en bloc* would distort relationships, and organs which had been dissected elsewhere and sent in for examination could not possibly yield information which would be useful in a study of the kind reported here.

I am concerned with the re-implication of abnormality of the thymus as a cause of sudden death, because when it was accepted it was used only as a mask for the ignorance of those pathologists who were inept at making accurate examinations, or to protect the pride of those who were unwilling to admit, after the autopsy, that they did not know the cause of a death.

(Dr. Carr) It is a pleasure to have started the first fight of today. I am complimented that I leave at least two people cold. No one, in fact, seems to be very hot about the subject. I should like to answer these questions in sequence, and in

answer to the first question, may I say that we agree with Dr. Werne's statement about anomalies, but wish to note that congenital anomalies of the circulatory system, particular of the coronary vessels and great vessels of the neck, are far more common with the thymic status than in normal babies. His second suggestion that normal infants have large thymuses is also true, and it is our experience that the thymic size in babies is actually far larger than the published figures. The thymic weight also varies with the baby's nutrition, with its heritage, and with geographic distribution, but the doctor missed the point entirely, because size actually has nothing to do with auricular compression, nor does size alone have to do with death in status thymico-lymphaticus. Large thymuses may be large and compatible with normal function. They may also be large and associated with other related changes, which cause death. As far as the figures he cites are concerned, and his suggestion that we should do autopsies on normal babies to find out the thymic size, may I state that we do many autopsies on normal babies and find large thymuses. We also do autopsies on babies who have died by accident, who have large thymuses. But, may I call your attention to the fact that these babies that I have reported had large thymuses and no accidents, and that they are still dead. I would suggest that he do more autopsies on babies which do not die by accident, to find the normal size under other circumstances. We cannot agree at all with the statement that thymic hemorrhages are commonly found in routine autopsies in babies dying of accident or disease. We would be very happy to match figures with Dr. Werne, because we have, in autopsies on 1500 babies, 135 cases of this sort. As far as Dr. Dutra's criticism of the selection of cases is concerned, we are quite conscious of the quality of our material, and agree that material sent in from the outside is the worst kind of material we can get, but we chose it specifically for this initial presentation and reopening of this subject, because it left us personally out of the picture, and as I said before, eliminated the geographic imputation to some degree.

**HISTOPATHOLOGIC DISTINCTIONS BETWEEN ACTUAL AND ALLEGED ASPHYXIAL DEATH DURING INFANCY.** Jacob Werne and (by invitation) Irene Garrow, New York, N.Y.

*Abstract.* In a previous investigation of 167 consecutive infants under 1 year of age found dead in crib, carriage, or bed while in apparent health, the authors found, as the most common cause of death, fulminating respiratory disease. The sudden death in these cases is not due to accidental mechanical suffocation but represents a further development of the circulatory collapse that not infrequently characterizes severe infections. Since such cases are ordinarily still certified as due to accidental mechanical suffocation, a comparison of the lesions found in this group with those encountered in infants dying of known asphyxial causes is reported. Fifteen consecutive cases, 1 month to 4 years of age, found dead of drowning or strangulation, failed to show significant tracheobronchitis, otitis media or mastoiditis, or interstitial pneumonitis, and showed relatively slight congestion, edema, and hemorrhages of the lungs as compared with the group allegedly suffocated by bedclothes. Also conspicuously absent in the known asphyxial deaths were the extreme visceral congestion and foci of cellular infiltration and parenchymatous degeneration which we have encountered in infants dying of fulminating respiratory disease.

**AIR EMBOLISM AS A COMPLICATION OF LUMBAR PNEUMO-ENCEPHALOGRAPHY. REPORT OF TWO CASES WITH NECROPSY FINDINGS.** Frank C. Coleman, John R. Schenken, and Walter D. Abbott, Des Moines, Iowa, and Omaha, Nebr.

*Abstract.* Two cases of fatal air embolism are reported as a complication of lumbar pneumo-encephalography. Symptoms began in each case near the com-



pletion of the procedure, and death followed within a few minutes. Two factors seem to be responsible for the condition: (1) canalization of an epidural vein, and (2) the pressure of the injected air must be greater than the venous pressure.

### Discussion

(Dr. Walter Putschar, Charleston, W. Va.) We have seen a case similar to those presented during an encephalographic procedure. The child was in bed, air was being injected, and the child died with a stoppage of the heart under continued respiration. In this case the spinal cord was removed and we could not demonstrate any openings in the veins. However, we assumed that in some way the needle must have gotten into the venous plexus. The heart was distended with air, and there was no doubt that it was an example of fatal air embolism.

SOME SIGNS, FINDINGS, AND INTERPRETATIONS OF CRIMINAL ABORTION. William E. B. Hall, St. Louis, Mo.

*Abstract.* 1. Massive hemolysis following hemolytic abortifacients. Three cases were studied in which ingestion of abortifacient liquids was followed by massive hemolysis with the production of hemoglobinuria and red-stained saliva, tears and perspiration, the latter copiously staining clothing, sheeting and pillows, ultimately followed by death in approximately 2 days. A listing is made of liquid abortifacients capable of causing hemolysis by ingestion, absorption, or parenteral injection and sometimes by intra-uterine injection.

2. Bacteremia in the embryo and placenta. Bacteria demonstrated in ample quantities in the vessels of early embryos are interpreted as indicating traumatic damage to the chorio-amniotic sac with entry into the fetal vascular system prior to death. As such their presence is a presumptive sign of an infected abortion produced by pre-abortifacient interference.

3. Postabortion uterine myiasis. A case of abortion is reported in which autopsy showed the uterine fundic cavity to be occupied by a number of second stage larvae of the blue bottle fly. Their discovery was in corroboration of the production of abortion by the instrument used by the patient—a stick obtained from the barnyard manure pile. Further, the identity of the larvae and their stages of development served as excellent indicators of the approximate time of abortion.

### Discussion

(Dr. Frederic Wohlwill, Hathorne, Mass.) Regarding the last item, I would like to emphasize that in these cases we deal with a fetal septicemia with infected emboli in the capillaries of the chorionic villi, leading to an "intravillous placentitis." As has been mentioned, the fetus will, in the majority of cases, be expelled before the mother has been infected. But when we studied this affection in 1928 we found that in 2 cases in which *Eberthella coli* was the agent the mother had been secondarily infected. In one of these she died of *E. coli* septicemia.

PATHOLOGY OF ACUTE CARBON TETRACHLORIDE TOXICITY. Henry D. Moon, San Francisco, Calif.

*Abstract.* This study was based on 12 fatal cases of acute carbon tetrachloride poisoning resulting from inhalation or ingestion. In 11 of the 12 cases there was associated acute or chronic alcoholism. The duration between poisoning and death varied from 4 to 18 days, thus affording an opportunity to study the morphologic changes in the liver and kidney at various intervals following poisoning. The hepatic lesions were characterized by early severe central necrosis of the lobules followed by an inflammatory reaction and regeneration which occurred almost simultaneously. The renal lesions in patients dying within a few days of poisoning were relatively slight and the pathologic changes were those of moderate cloudy

swelling. In the later stages tubular degeneration was profound and resembled the picture seen in the kidney of the "crush syndrome." Pulmonary edema occurred clinically as a late manifestation and was present in nearly all cases at autopsy. A diagnostic triad of central lobular necrosis of the liver, renal tubular degeneration, and pulmonary edema was consistently present in this series of patients.

#### *Discussion*

(Dr. Alfred Angrist, Jamaica, N.Y.) I should like to ask about the relationship of alcoholism to carbon tetrachloride poisoning. In the 2 instances I have seen, chronic alcoholics who did not stop taking alcohol succumbed more rapidly to exposure to carbon tetrachloride than did normal individuals without a history of alcoholism. I understand from your chart, Dr. Moon, that you found several such cases. The point is of considerable industrial importance because any patient who has a history of alcoholism should not be exposed to this hazard. There is a peculiar feature about this association of alcoholism and carbon tetrachloride poisoning in that men who have that type of history tend to select this type of trade because they find they can get a cheap "drunk" with very little liquor.

(Dr. Moon) I believe there is a close relationship. I have looked at a great many kidneys specifically with that in mind, and there is cloudy swelling of the proximal convoluted tubules in the kidneys of both acute and chronic alcoholics. There are also, in some cases, binucleated epithelial cells and mitotic figures in the proximal convoluted tubules.

**POST-MORTEM BIOCHEMICAL CHANGES.** Walter W. Jetter and (by invitation) Regina McLean and Mary K. Nutter, Boston, Mass.

*Abstract.* A large portion of all medicolegal autopsies are performed on bodies of persons found dead and concerning whom there is little or no reliable anamnestic data. Every reliable source of information likely to shed light on the nature of the terminal illness or on the cause of death may need to be explored. An investigation of the chemical constitution of blood removed after death from a group of persons whose deaths were sudden and from causes least likely to have resulted in ante-mortem biochemical abnormalities was undertaken with two objectives. One was to assess the potential usefulness of such determinations in estimating the probable length of the post-mortem interval. The second was to determine the extent to which agonal or post-mortem changes might mimic biochemical abnormalities caused by disease.

Post-mortem samples of blood from the right heart were obtained from 20 persons. The interval between death and sampling varied from 1 to 69 hours. In all but 2 instances the bodies had been kept at refrigeration temperature for most of the post-mortem interval. In only one instance was there obvious putrefaction or hemolysis of the blood sample. Whole blood and plasma were analyzed for pH, carbon dioxide, glucose, non-protein nitrogen, and various electrolytes.

Certain changes in pH, lactic acid, plasma potassium, and inorganic and organic acid-soluble phosphorus were related to the length of the post-mortem interval. Generally speaking, the greatest change observed was that which had taken place during the agonal or early post-mortem period. All but one of the observed pH values were lower than any found in the living organism. The average for the first 12 hours was 6.79, while that for the second 12-hour period was 6.43. There was an irregular rise in plasma lactic acid during the first 12 hours. All values for plasma and cells were greatly elevated above normal. There was an immediate rise in plasma potassium which also increased irregularly with time. The cells showed a significant loss of this constituent. Inorganic phosphorus appeared in the cells at the same time that there was a great decrease of cellular organic acid-soluble phosphorus. Simultaneously the plasma contained a small amount of organic acid-

soluble phosphorus and a two-fold to nine-fold increase of inorganic phosphorus. The greatest increase was noted in the second 12-hour period. Plasma sodium was not significantly changed. The cells of 14 cases contained this electrolyte. Of the plasma calcium values, 11 were normal, while 5 were slightly elevated. Five cases showed significant invasion of the cells by calcium. Plasma magnesium was increased in 8 instances, decreased in one, and normal in the rest. Cell magnesium was increased. Cell chloride was generally increased, while in all but 3 cases plasma chloride remained normal or slightly decreased.

The carbon dioxide content of whole blood protected against loss of gas remained essentially normal. There was a substantial decrease in the carbon dioxide-combining power of plasma. A second series of cases showed the normal values for carbon dioxide content in whole blood to be due to greatly increased carbon dioxide tension. Non-protein nitrogen values varied between 28 and 124 mg. per cent. Glucose values ranged from 33 to 486 mg. per cent.

**CHANGES OF EFFUSED BLOOD OUTSIDE AND INSIDE OF THE BODY: THEIR SIGNIFICANCE FOR LEGAL MEDICINE.** George Strassmann, Waltham, Mass.

*Abstract.* After effusion of blood, changes in the hemoglobin occur outside or inside of the body. These changes were studied on human and animal material. If blood dries outside of the body, methemoglobin or hematin is formed after some time. In watery solutions the characteristic spectrum remains visible for weeks. The easiest method for identifying small and old traces of dried blood is the demonstration of hemochromogen crystals, which also give a characteristic spectrum. Crystals and spectrum of hemochromogen are similar in animal or human blood. Heating with a mixture of sodium hydroxide and pyridin gives good results.

Inside of the body phagocytic cells change the hemoglobin to hemosiderin and to hematoidin. After injections of homologous or heterologous blood in rabbits and mice, hemosiderin was found earlier than in spontaneous or traumatic cerebral hemorrhages of men. In many older cerebral hemorrhages of men or animals no hematoidin but only hemosiderin was discovered. The reason for the absence of hematoidin in such cases is doubtful. The presence of phagocytes with blood pigment is evidence that hemorrhage has occurred some time before death. The time interval between the onset of the hemorrhage and the first appearance of hemosiderin and hematoidin varies for different animals and men, and for different tissues and organs. In the brain the formation is slower than in other tissues. Perivascular phagocytes in the brain are indicative of a former hemorrhage which can be of traumatic or spontaneous origin. They are especially frequent in general paresis and cerebral arteriosclerosis. Phagocytes with the blood pigments remain in the hemorrhagic area for an indefinite period of months and years. In cerebral hemorrhages of man hemosiderin was not found before the 5th and 6th day, hematoidin not before the 11th to 14th day.

The stable combination of carbon monoxide with hemoglobin makes possible the detection of carbon monoxide in blood or bloody fluids from exhumed bodies in acute carbon monoxide poisonings, but outside of the body carbon monoxide-hemoglobin disassociates earlier and could not be detected in effused blood from fatal gas poisonings in animals after a few weeks.

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**INTRAMEDULLARY TUMORS OF THE SPINAL CORD AND FILUM TERMINALE.** James W. Kernohan, Rochester, Minn.

*Abstract.* We have found in our series of 856 tumors of the spinal cord that approximately one-quarter arise from the spinal cord itself or the filum terminale. About 90 per cent of these lesions are gliomas, similar in most respects to gliomas

of the brain. In contrast to brain tumors, however, the majority of the intramedullary tumors are ependymomas, and in the filum terminale practically all tumors are ependymomas. The association of intramedullary tumors with syringomyelia is remarkably high.

### *Discussion*

(Dr. Henry Edmonds, Washington, D.C.) May we hear from Dr. Kernohan concerning his ideas of the mechanism of syringomyelia in association with neoplasm?

(Dr. Bernard Black-Schaffer, Durham, N.C.) Since gliomas differ so markedly from the extra-nervous epithelial and mesothelial tumors, I should like Dr. Kernohan to comment further on his criteria for classifying the gliomas numerically as 1, 2, 3, 4.

(Dr. Kernohan) In regard to the first question, I am not sure that I can say what the association between syringomyelia and the neoplasm is. It is interesting that 62 per cent have associated tumor and syringomyelia. I rather think they are independent phenomena.

In regard to the question of malignancy, I have only about 3 minutes and it would take a little longer than that to explain this, but I did answer it somewhat on the lantern slides during my presentation. I pointed out that astrocytoma grade I is the same as the original astrocytomas described by Bailey and Cushing. The grade II astrocytoma has more cells, has pleomorphism and some hyperchromatic nuclei, but no giant cells, no mitotic figures, and the blood vessels are well formed. In astrocytoma grade III there is greater cellularity, more pleomorphism, some giant nuclei, and an occasional giant cell. There are only a few mitotic figures, only slight proliferation of the endothelial cells of blood vessels, and some proliferation of the adventitia of the blood vessels. In astrocytoma grade IV the tumor is very cellular, pleomorphism is marked, giant cells are usually numerous, and there are many mitotic figures. Proliferation of both the endothelial and adventitial cells of the blood vessels is marked and areas of necrosis are numerous. The same criteria apply to ependymomas and we have not yet evaluated our oligodendrogliomas. I hope this answers the second question.

EPIDERMOID CARCINOMA OF THE SKIN OF THE UPPER EXTREMITIES. Richard E. Johnson (by invitation) and Lauren V. Ackerman, St. Louis, Mo.

*Abstract.* Epidermoid carcinoma of the skin arising from the dorsum of the finger of the hand, or from the forearm, has individual characteristics and the rate of metastasis bears relation to certain pathologic findings. Seventy-two histologically proved consecutive tumors occurring in 71 patients were analyzed in detail. These patients were followed at least 3 years; they were outdoor workers (almost exclusively farmers) with histories of many years' exposure to the elements. In no instance was there an old scar, draining sinus, or arsenical dermatitis as an etiologic agent. The rate of metastasis in the entire group was 15 per cent, with an absolute 3-year cure rate of 59 per cent. The high death rate of 27 per cent during the 3-year follow-up period (without evidence of carcinoma) was attributed to the advanced age of the patients. Twenty-two per cent were over 80 and 49 per cent were over 75 years. The development of metastasis was contingent mainly on two factors: previous inadequate treatment, and the stage of the primary tumor. Of 24 previously treated tumors, 3 had metastasis on admission and 5 showed subsequent metastasis. Of 48 untreated tumors, 3 had metastasis on admission and none exhibited late metastasis.

Two distinct stages of tumor growth can be identified by the depth of invasion relative to the coiled portion of the sweat glands. All metastasizing tumors in

which the depth could be determined had extended below the sweat gland level. In the 24 previously treated tumors, 15 were below the level of the coiled portion of the sweat gland and 3 were indeterminate. Seven of the 15 and 1 of the group of 3 were found to have metastases. Of the 48 untreated tumors, 17 extended below the sweat gland level and 10 were indeterminate. Only 1 of these 17 and 2 of the group of 10 had metastases. Thus it is emphasized that previous inadequate therapy and depth of invasion are the most important factors in relation to the spread and prognosis in any group of these neoplasms.

A clear-cut division in two stages, above and below the coiled portion of the sweat gland, is easily explained. There is considerable resistance to invasion when the carcinoma meets the zone of degeneration of connective tissue fibers in the dermis. This layer of degenerating connective tissue in the dermis is consistently present, due perhaps to chronic exposure to sunlight, and natural ageing. In the presence of tumor it is progressively replaced by proliferating neoplasm and granulation tissue. This sequence of events results in a mass several times the volume of the original layer of degeneration. For a considerable period of time the growth capacity of the tumor is expended in replacing this area of degeneration and since the sweat glands arise just below this zone, they constitute an excellent manner of staging the tumor in terms of breaching of the "degeneration zone barriers."

INVOLVEMENT OF THE STOMACH IN MALIGNANT LYMPHOMA. H. R. Wahl and (by invitation) J. H. Hill, Kansas City, Kan.

*Abstract.* Five cases of malignant lymphoma in which necropsy revealed unusual macroscopic infiltration of the stomach are presented. The infiltration, chiefly in the submucosa, produces two distinctive gross anatomic patterns. One is characterized by multiple discrete nodules with or without ulceration. The other is characterized by diffuse infiltration of the submucosa leading to the formation of giant rugae. In all 5 cases infiltration of the region of the antrum was minimal or absent. Except for fatal hemorrhages from the ulcerated nodules in a case of Hodgkin's sarcoma, no clinical signs or symptoms could be attributed to the extensive lymphomatous infiltration in these cases. It is believed these two gross anatomic patterns indicate involvement by malignant lymphoma.

#### *Discussion*

(Dr. Lauren V. Ackerman, St. Louis, Mo.) We have been impressed by the rather atypical clinical evolution of some of the lymphosarcomas of the stomach. In this group 5-year survival occurred rather frequently even with poorly conceived radiation and surgical therapy. I wonder if Dr. Hill would comment further on the evolution of lymphosarcoma of the stomach as compared with lymphosarcoma in other areas.

(Dr. William L. Robinson, Toronto, Canada) I was interested in the distribution of these lesions. They appear to be confined almost wholly to the fundic end of the stomach, and I wonder if Dr. Hill has made any attempt to localize these lesions, whether he has determined where the fundopyloric line is as compared to the localization of these lesions.

(Dr. Hill) We have in our material no satisfactory cases of primary lymphosarcoma of the stomach. I think this diagnosis is very difficult to establish. The surgeon may think he has a primary tumor only to find at necropsy that is not the case. Since we do not have such material, I cannot make any comments. I believe it may occur, but it must be very rare.

The distribution of the lesions in the stomach is of considerable interest. We do not understand why the infiltrations tend to occur in the fundus and body and not in the antrum. There are several cases of this same general type in the litera-

ture in which the prepyloric region is more extensively involved than the remainder of the stomach. Certainly the antrum contains lymphoid tissue and if these patients lived long enough I think they might all eventually have involvement of the antrum.

**BENIGN LYMPHOID POLYPS OF THE RECTUM.** Elson B. Helwig and (by invitation) James L. Hansen, Washington, D.C.

*Abstract.* Among the polypoid tumors of the rectum observed at the Army Institute of Pathology were 70 examples in which the lesion was comprised of lymphoid tissue. About one-half of these lesions were originally diagnosed microscopically as benign lymphoma, lymphoid polyp, heterotopic lymphoid tissue, and hyperplastic lymphoid tissue. The other half were diagnosed as giant follicle lymphoma, lymphosarcoma, malignant lymphoma, Hodgkin's disease, and reticulum cell sarcoma.

An analysis of the material contained in both groups showed a similar gross and histologic pattern. The lesions were polypoid, sometimes pedunculated and sometimes sessile, and generally, but by no means always, single. The surface was usually smooth and gray, but sometimes convoluted. The clinicians readily differentiated the lesion from a hemorrhoid, but often mistook it for an adenoma. The polypoid masses varied in size from 2 to 40 mm. Microscopically, the polyps were comprised of lymphoid tissue which exhibited a pattern of primary follicle formation with so-called germinal centers. The primary follicles as well as the germinal centers tended to be large and in many instances were of irregular shape. On the basis of the arrangement of the follicles a lobular structure was usually apparent, with the lobules of lymphoid tissue separated by a generally delicate connective tissue and reticular framework. Silver stain for reticulum showed a moderate amount of reticulum between the lobules of lymphoid tissue but only a sparse distribution within the lobules. Peripheral or central sinusoid structure, as found in normal lymph nodes, was absent. The lymphoid cells either in lobular formation or as ill-defined foci sometimes replaced the normal glandular mucosa.

General clinical examinations failed to disclose a single instance in which there were signs of any type of lymphoblastoma. Many of the examinations were particularly thorough because of the original diagnosis of lymphoblastoma. About one-third of these patients have been followed for several years and at this time none has developed generalized lymphoblastoma of any type. All but one patient, who received X-ray therapy, were treated by simple excision. On the basis of these observations polypoid lymphoid tumors of the rectum showing a pattern of primary follicles with germinal centers are to be considered benign. The structure of the lesions suggests that the change is one of hyperplasia.

#### *Discussion*

(Dr. Walter Putschar, Charleston, W. Va.) We have seen 4 cases of this type, in all of which the lesions were located at the mucocutaneous junction of the rectum. One is very much impressed by the features shown by the lesion which make one wonder if it is really a hyperplasia, or does it go back to the normal histologic structure which is exaggerated? It is a fact that some of the mammals, particularly the dog, have lymphoid tissue exactly in this area, and there may be a phylogenetic element in the formation of these lesions. All the lesions we have seen have remained benign, for as long as 11 years. All of them showed part of the squamous epithelium. None of the cases showed any inflammatory lesion of the rectum which would make inflammatory hyperplasia likely.

(Dr. Helwig) I am aware of what Dr. Putschar has pointed out, that in certain ruminants there is lymphoid tissue in the area of the mucocutaneous junction. In an attempt to see what type of inflammation might produce this lesion we

studied 45 cases of schistosomiasis, syphilis, lymphopathia venereum, tuberculosis, and of non-specific granulomas involving the rectum and anus, and in none was there an appreciable collection of lymphocytes. The one lesion in which occasional lymphoid follicle formation occurs is in apparently non-specific inflammation of the anal crypts. In this collection of lymphoid polyps we saw a portion of a crypt once in a while, but it seems doubtful that the entire group of lesions is associated with a non-specific cryptitis.

**BENIGN LYMPHOMA OF RECTUM.\*** Elwyn L. Heller and (by invitation) Homer H. Lewis, Jr., Pittsburgh, Pa.

*Abstract.* The clinical and pathologic features of 9 cases of benign lymphoma of the rectum are presented, with a review of the appropriate literature. The importance of the lesion lies in the recognition of its benign characteristics in spite of histologic features simulating malignancy. In all probability the lesion is much more common than the occasional references would indicate.

#### *Discussion*

(Dr. Joseph E. Pritchard, Montreal, Canada) I would like to ask Dr. Heller if these tumors are ever multiple. They are usually solitary. All that I have seen have been strictly solitary. The question is important because I think sometimes the surgeon presumes there are other polyps there because of the polypoid nature of the tumor.

(Dr. Heller) In our series the majority of these cases were solitary, but multiple involvement has been described. I believe Dr. Helwig just stated that 60 per cent of his lesions were solitary, and I assume that 40 per cent were multiple.

**A STUDY OF THE HISTOGENESIS OF ENDOCERVICAL METAPLASIA AND INTRA-EPI-  
THELIAL CARCINOMA.** Lee Howard (by invitation), Cyrus C. Erickson, and  
(by invitation) Leland Stoddard, Durham, N.C.

*Abstract.* The microscopic lesion of the cervix uteri that is known as intra-epithelial carcinoma, carcinoma *in situ*, or pre-invasive carcinoma, has been the subject of varied interpretation. Disagreement has concerned its site and mechanism of origin and its relation to invasive carcinoma. We think the available published data do not indicate clearly that this surface lesion inevitably becomes an invasive lesion. Continuing critical study and contributing observations are deemed preferable to unqualified acceptance of the inexorably malignant progression of this lesion. The present preliminary report records histologic observations on 400 total cervixes and deals with the histogenesis of endocervical metaplasia and intra-epithelial carcinoma. Only 3 per cent were removed because of clinically observed cervical lesions; none was considered carcinoma. The mean age was 43 years. Only 6 pregnant uteri were present in the group. In each case, the entire cervix was cut into blocks and at least 2 sections made from each block so that 6 to 24 sections per case were examined.

Observations regarding the histogenesis of endocervical metaplasia agree with those of Carmichael and Jeaffreson and of Auerbach and Pund. Proliferation and differentiation of basal or reserve cells was followed from a thickness of one cell to the development of a complete surface layer. Such a differentiated, metaplastic lining could be distinguished from the normal squamous epithelium of the portio vaginalis. Some degree of metaplasia was present in 83 per cent of cervixes. In an additional 1.8 per cent of cases, which included one of the 6 instances of pregnancy, cytologic atypicalities in metaplastic epithelium ranged from minor diver-

\* This article will appear in a subsequent issue of *The American Journal of Pathology*.

gence to extensive cytologic variation simulating intra-epithelial carcinoma. Intra-epithelial carcinoma was found in 3.5 per cent of cases.

It was possible to trace the proliferation of reserve cells through all stages of metaplasia to instances with cytologic variability and atypicality of sufficient degree to constitute the lesion termed intra-epithelial carcinoma. The great frequency of metaplasia compared with that of intra-epithelial carcinoma indicates that this progression is far from inevitable in a given case. The histogenesis explains the dominant location of intra-epithelial carcinoma, namely, the endocervical canal. In one case atypicalities extended onto the portio vaginalis, and, in a few instances not included in this report, lesions appearing in the portio vaginalis have been seen. The latter would seem to stem from a different histogenetic mechanism. No correlation of lesions with pregnancy or endocrine disturbances was possible. No instances of invasive carcinoma were uncovered in this study. Further observations concerning the potentiality of intra-epithelial carcinoma are required.

### *Discussion*

(Dr. N. Chandler Foot, New York, N.Y.) The matter of intra-epithelial carcinoma is a very important one which is much to the fore at present. It is one which will need a great deal of study because in connection with the growing use of exfoliative cytology a great many intra-epithelial lesions are turning up. The question is, are these lesions reversible or are they not? We shall have to follow up all of these cases in order to answer that question. Whether they are reversible or not is difficult to say, but it is wise on the part of these authors to divide the intra-epithelial carcinomas and the metaplasias with atypicality. There is a dividing line there which is very tenuous, and it is difficult to say where one type goes over into the other. The cases of atypicality in the cervix with metaplasia will yield a great number of dyskeratotic cells which Papanicolaou calls "Birkhead" cells. These are always very difficult to interpret, and we find that in about half the cases they do not necessarily mean cancer, whereas in the other half intra-epithelial carcinoma is present. We must also be careful not to be didactic about intra-epithelial carcinoma and try to subdivide that one narrow classification into sub-heads, as some are doing at present. I think that that is going too far. In indicating the broadness of the subject I would like to point out that Bowen's disease of the skin, epidermoid carcinoma in the esophagus, and even carcinoma of the prostate show such intra-epithelial lesions, so that we must study a great deal more than these carcinomas of the cervix, and we shall have to follow them up very carefully in order to determine whether intra-epithelial carcinomas are true carcinomas that will necessarily go on to invasion. A great many of them do, and it seems likely that this is the very beginning of carcinoma, although we cannot be sure of it.

(Dr. Samuel R. Haythorn, Pittsburgh, Pa.) I have been interested in squamous cell metaplasia for a good many years, and I do not believe it is possible to take any set of lesions and trace squamous cell metaplasia into cancer. I think that there are two kinds of metaplasia, and one is primarily cancer from the beginning, and the other is a reversion to a lower type, or more protective type, of epithelium which is probably a part of a reparative process. While it is conceivable that the area might be involved with other tissues at the site of a cancer, it should not be considered a necessary step in cancer development, and in that sense it is reversible as Dr. Foot has said. Once this second type of metaplasia has occurred it does not follow that cancer is an irrevocable sequence.

(Dr. William L. Robinson, Toronto, Canada) We have been making a study of these cases also, and I was rather surprised that Dr. Howard and his co-work-



ers have not stressed one feature in metaplasia, the staining of the cytoplasm. I feel that carcinoma *in situ* gives a cell of more basophilic type as well as these other atypical characteristics, and as I was taught by Mallory, this and the loss of polarity of cells are the two distinguishing characteristics of malignancy. I would like to stress the basophilism of these cells.

(Dr. J. F. A. McManus, Birmingham, Ala.) Concerning the staining reaction of carcinoma *in situ*, the basophilia is apparently related to the increase in the ribonucleic acid in the cytoplasm. Dr. Findley and I have found that carcinoma *in situ* lacks the glycogen of the ordinary epithelium of the portio vaginalis, of the squamous variety. We have been interested in this matter of squamous metaplasia, repeating some of the work of Jeaffreson and Carmichael. We came to about the same conclusion as reported in this paper. Under certain conditions atypicality can appear in squamous metaplasia. There are metaplastic areas in which small droplets of mucin-like material are seen in the metaplastic cells of the endocervical glands, and others in which the mucin has disappeared. I am not sure that histologically one can suggest whether areas of squamous metaplasia are going to remain benign or become malignant.

(Dr. Joseph Tannenberg, Batavia, N.Y.) I like to emphasize in this connection the limitations of our morphologic observations. We have been concerned with these lesions for many years, and the more we see of them, the more we are compelled to state the impossibility in many cases of foreseeing whether these atypical cells will later become malignant tumor cells. There is only one thing to do: state what we see, and request observation of the patient in which such lesions are seen. We cannot foresee whether what appears to be metaplasia today will be a carcinoma in 6 months. We have to see new specimens eventually at monthly intervals, and repeated vaginal smears might be a helpful accessory in these cases. I would like to point out that we see the same lesions in caruncles and wherever squamous and columnar epithelium meet, if there is some kind of chronic inflammation. Thus I feel we have to emphasize the limitations of a single morphologic examination and have to see repeatedly, in 3 and 6 months, eventually, new biopsies from these patients if clinical examination shows suspicious lesions. I think we all have had occasion to see pitfalls of a single histologic examination when carcinomas have developed in patients who failed to see their physicians for a year or two after metaplastic lesions were seen, but hundreds of those patients will never develop carcinoma.

(Dr. Shields Warren, Boston, Mass.) This very careful study of Dr. Howard and his associates is the sort of thing I hope will be carried on widely. One of the chief values of the cytologic technic has been to emphasize the initial lesions that sometimes shed cells, and I believe that perhaps a great deal of the value of the method will be through the attention that it has called to these early and previously not too greatly noticed cervical lesions. One of the things that struck me in Dr. Howard's statistics is the same type of thing that we have found in our own statistics: the relatively high proportion of intra-epithelial carcinoma. That certainly is compatible with what Dr. Foot has pointed out as indicating a relationship with Bowen's disease of the skin. An incidence of 6 per cent runs far too high to permit this lesion to be a simple inexorable progression to carcinoma. I think it is one of the very real jobs of those interested in the cervical epithelium to attempt to evaluate in the next few years the abnormal epithelial lesions of the cervix which remain relatively static and those which progress to carcinoma. We have in Dr. Howard's series 3.5 per cent of intra-epithelial carcinomas, and I think it would be impossible to assume that these lesions are always precancerous unless some remain static or fairly static for long periods of time, or some of them re-

gress. Otherwise, we ought to have a tremendously greater incidence of cervical carcinoma in the female population than we have.

(Dr. Alfred Angrist, Jamaica, N.Y.) In this problem I would like to have the term "observation" defined. If these cases do have the possibility of developing malignancy, they survive a much longer period of time than the average early fully evolved carcinoma would suggest. Many of these may not go on to carcinoma in the ordinary sense. The term "observation" is what is variable and misleading. When this diagnosis comes forth from the laboratory, in some institutions the patient is immediately subjected to a major operative procedure. They do everything for this lesion from a radical Wertheim procedure, a panhysterectomy, or a simple hysterectomy to local excision, conization, or cauterization. How are we ever going to find out about the behavior of this lesion unless somebody has the courage to let these patients actually be observed to see what happens? I am anxious to know what you mean and recommend by the term "observation." Unless we have better standards as to the meaning of it, we will never know where we are at.

(Dr. Howard) I am afraid I will not be able to reply specifically to all of the questions and comments that were made. I am pleased that most of those discussing this paper would seem to agree in some respects with what I have said.

First, in regard to Dr. Foot's comments: I agree in believing that the follow-up in these patients is the important thing in developing what is the actual significance of this lesion.

In regard to the second comment, we feel that histogenetically the lesions we have shown are derived from the same cell, that is, the reserve cell. I think that in some instances the proliferation of this cell is perhaps along the lines of intra-epithelial carcinoma from the very beginning, and in other instances, it develops into what we call simple metaplasia, and perhaps stays there indefinitely.

In regard to the staining characteristics, I was not using color photomicrographs, but I have observed that in general the cytoplasm in metaplasia is of a pink character and in the intra-epithelial carcinomas it is of a more bluish color. We have not studied mucin preparations.

In regard to the follow-up, I think I can answer several of the questions at one time. By "observation" I mean follow-up of the individual case by repeated biopsies. These patients should be observed carefully and frequently by competent gynecologists. We have been fortunate in obtaining the cooperation of the gynecologic service and have been able to follow some of these patients over a long period of time. For example, we have seen several cases in which the lesion was present during pregnancy, and have followed the patient through three or four subsequent pregnancies with no change in the character of the lesion, so I think that the fundamental thing is to convince the gynecologists that the patients should be followed carefully and frequently by biopsy.

GENESIS OF CARCINOMA OF THE ENDOMETRIUM. Arthur T. Hertig and Sheldon C. Sommers (by invitation), Boston, Mass.

*Abstract.* A series of 400 cases of endometrial carcinoma has been reviewed in an effort to collect factors significant in the genesis of this neoplasm. Thirty-one cases were collected in which endometrial specimens had been taken from 1 to 23 years prior to diagnosis of carcinoma. Biopsy material taken 15 or more years before cancer diagnosis was normal, but at shorter intervals, biopsies were usually abnormal. The shortest interval between a normal curettage and carcinoma was 3 years. Cystic hyperplasia and endometrial polyps occurred in several cases, and were comparatively frequent 6 or more years before carcinoma. Adenomatous hy-

perplasia, that is, small outpouchings of endometrial glands with reduplication of lumina, and cellular anaplasia or de-differentiation, were relatively most frequent from 4 to 9 years before carcinoma. None of these changes are considered irreversibly neoplastic because of cases reverting to normal after ovulation. Carcinoma *in situ*, consisting of large pink cells with pale nuclei arranged in glands and often sharply demarcated, is the earliest lesion considered true neoplasia. Found up to 11 years before diagnosis of invasive carcinoma, it was relatively most frequently observed 4 to 5 years before the invasive stage. The 31 cases collected have essentially the same age distribution and mortality as found among the 400 cases, and are believed to form a representative group. They are unusual in that previous curettage in all 31 cases and radiation therapy in 19 of these cases did not prevent the development of cancer, since hyperplastic precancerous lesions presumably are more easily destroyed than invasive carcinoma.

#### Discussion

(Dr. Jacob Werne, New York, N.Y.) Those of us who see surgical pathologic material are asked what to do about the lesions presented here as precancerous. I wonder if the authors can tell what proportion of their cases showing these changes failed to develop carcinoma.

(Dr. Sommers) There were approximately an equal number of cases to the 32 with invasive carcinomas which in the final analysis we decided to discard from our series because of borderline or insufficient evidence of fully invasive carcinoma. The subject is being studied from the biopsy point of view. I would not be able to hazard a guess as to how frequently anaplasia and hyperplasia fail to go on to carcinoma.

**HISTOCHEMICAL STUDIES ON ALKALINE AND ACID PHOSPHATASE IN RENAL CARCINOMA OF THE FROG.** Gang Young (by invitation), Charles Breedis, and Balduin Lucké, Philadelphia, Pa.

*Abstract.* The common occurrence and availability of a variety of tumors in cold-blooded vertebrates permit investigation of their biochemical properties. Through such studies it may be hoped to broaden the scope of knowledge concerning the general characteristics of neoplasia. It was the aim of the present study to determine the activity of alkaline and acid phosphatase in relation to the development and growth of the carcinoma which commonly occur in the kidney of the leopard frog, *Rana pipiens*. The enzymes were chosen because they are known to be present in the normal kidney of the frog. Using a slightly modified method of Gomori, it was found that during the growth of the frog carcinoma, from minute localized nodules to large invasive and metastasizing tumors, profound changes occurred in enzyme content of the neoplastic cells. As the tumors became larger and more aggressive, alkaline phosphatase activity diminished and in many of the larger tumors disappeared entirely. In contrast, acid phosphatase activity increased until this enzyme became very abundant in the growing tumor. Metastases from the primary tumor, occurring relatively late in the course of neoplastic development, had a pattern of phosphatase distribution similar to that of the larger primary tumors. These results led to the conclusion that the activity of both acid and alkaline phosphatase becomes greatly altered, the former notably increasing and the latter decreasing during the course of development of the frog carcinoma.

#### Discussion

(Dr. Shields Warren, Boston, Mass.) I think it is of interest that a variety of tumors are shown in animals as well as in man to have this shift in the phosphatase property as the cells take on malignant tendencies. We have been interested in a

somewhat similar investigation of the early stages of carcinoma of the stomach in man, and there a somewhat similar shift of the relationship of alkaline and acid phosphatase appears to occur as the epithelium changes to a carcinomatous type.

**HISTOCHEMICAL AND MICROCHEMICAL STUDIES OF RAT LIVER AFTER LOW PROTEIN DIET AND *p*-DIMETHYL-AMINO-AZOBENZENE.** R. E. Stowell and (by invitation) C. S. Lee and K. K. Tsuboi, Kansas City, Kans.

*Abstract.* Three groups of experimental rats were given the following diets: (1) normal synthetic diet, (2) low protein, low choline, high fat synthetic diet, and (3) comparable low protein diet with the addition of *p*-dimethyl-amino-azobenzene. Animals from each group were fasted 18 hours and killed at intervals of 6 weeks, 3 months, and 5 months, and after tumor formation. Liver blocks were fixed by various methods and stained for alkaline phosphatase, lipase, nucleic acids, glycogen, lipids, ceroid, mitochondria, and collagenous tissue. The relative changes in cellular constituents were measured quantitatively by the Chalkley ratio method. Liver samples from these same individual rats were analyzed chemically for content of water, lipids, nucleic acids, alkaline phosphatase, esterase, succinic dehydrogenase, collagenous tissue, and nitrogen and phosphorus in various fractions.

Chemical methods of measuring collagen do not show high correlation with cytologic volumetric measurements. Volumetric ratio methods for visible lipids agree reasonably well with the chemical observations. The mean volume of the liver cell nucleoli enlarges two to five times in cirrhotic stages, the nuclear volume doubles, but cytoplasmic volume changes less. Ceroid stains with methyl green and gives positive reactions for carbohydrate components by the Hotchkiss technic and Feulgen reactions with, as well as without, acid hydrolysis. Total nucleic acids are increased in the cirrhotic livers, but some of the increase in the desoxyribose type may be attributed to the increase in fibroblast nuclei. Alkaline phosphatase reactions are variable, but esterase is decreased to one-half and succinic dehydrogenase to one-fourth in both groups with cirrhosis. The decrease in proportion of tissue that is liver cell cytoplasm may partially account for this decrease in total enzymatic activity. Regenerating cirrhotic nodules show increased histochemical lipase reaction. The presence of *p*-dimethyl-amino-azobenzene in a diet low in choline and protein reduces evidence of choline deficiency. In early stages there is less fatty metamorphosis, ceroid, and collagenous tissue formation in the liver. It is important to recognize the possibilities of such coordinated research in the proper interpretation of cellular physiology in terms of pathology, histochemistry and microchemistry.

#### *Discussion*

(Dr. Alfred Angrist, Jamaica, N.Y.) I wonder if the authors noticed any change in the degree of polymerization in their studies, *i.e.*, if the size of the molecules of the nucleic acid fraction was studied from that standpoint, as by partial breakdown at different temperatures. In some work that was published we did find that there was a definite change in that direction, though total figures did not change as much as did the data obtained after partial hydrolysis or breakdown of polymers. This would indicate a definite difference in the size of the molecules in the hepatoma tumor fraction, in cirrhosis, and even in the liver tissue without the cirrhotic manifestation in animals on butter yellow. We found the same difference to occur in human livers with hepatoma and with cirrhosis.

(Dr. J. F. A. McManus, Birmingham, Ala.) There are several features of this study which appear extraordinarily interesting. One is this mosaic method of attack in which you have a number of technics giving different aspects of cellular metabolism, and another is the control of this study by comparison with the normal, which many of the histochemical reports appearing from time to time do not have.

In regard to the ceroid-like pigment, I think there are other pigments found in the tissues that have a carbohydrate component. Melanin under certain conditions does have some carbohydrate in it, and the same is true of the heart-failure pigment that we ordinarily call hemosiderin. There is a carbohydrate component in both of them.

I was interested in the histochemical demonstration of collagen. One of the technics needed most in histochemistry is something for protein. We have methods for carbohydrate and fat, but for the proteins, except the nucleoproteins, we do not have a satisfactory method. Some of the methods for amino acids determination may be used in the lower animals, but in the human being I do not know a satisfactory histochemical method for demonstrating protein. I was wondering which method Dr. Stowell is using for his collagen determinations.

(Dr. Paul Klemperer, New York, N.Y.) Is not the combined application of ultraviolet absorption spectrophotometry and controlled Feulgen reaction a definite indication for desoxyribosenucleic acid?

(Dr. Stowell) The study of the state of polymerization referred to in the first question would be interesting to investigate. Such an investigation was not undertaken because we were working with small samples, and we could not do everything with the samples used which in some instances were as small as 200 mg. of tissue. We hope we will eventually be able to take up other things of interest such as this.

In regard to Dr. McManus' comments, the chemical method for collagen used was that of Lowry, Gilligan, and Katersky (*J. Biol. Chem.*, 1941, 139, 795-804) as well as a comparison with the method of Spencer, Morgulus, and Wilder (*J. Biol. Chem.*, 1937, 120, 257-266). The first method was found to be more satisfactory from the chemical point of view, but we did not feel that either was as good as using a trichrome stain and the Chalkley ratio method for determining the volume of the collagen tissue. We hope that from this research we will get a better understanding of the relative advantages and limitations of various microchemical and histochemical methods. We are in the process of applying and evaluating the staining reactions for arginine, tryptophane, and tyrosin, and hope to get a reasonable indication of the distribution and amounts of these amino acids in tissues. In each of these animals the liver was separated into 4 fractions (acid soluble, alcohol-ether, nucleic acid, and protein) and the amount of nitrogen and phosphorus studied in each fraction.

In my remarks on ceroid I meant to show that ceroid gives reactions which might be easily misinterpreted as indicating that it contains desoxyribosenucleic acid if proper controls were not made. These histochemical technics are not so simple that you can read an article and go into the laboratory and blithely apply it to all kinds of tissue and assume you are measuring what you hope to detect. The Feulgen reaction when properly controlled is one of the best reactions we have for desoxyribosenucleic acid. Many people omit the proper controls. In studying nucleic acids by ultraviolet absorption I feel it is desirable to determine the absorption curve of tissues so one knows where the maximum absorption occurs. There are numerous substances which have an absorption at 260 m $\mu$  in common with nucleic acid, and I think it is hazardous to conclude that a photograph at one wave length constitutes a positive identification for nucleic acid.

THE EFFECTS OF A FOLIC ACID ANTAGONIST (AMINOPTERIN) ON ALBINO RATS. E. Woll (by invitation) and J. J. Oleson (by invitation), Burlington, Vt., and Pearl River, N.Y.

*Abstract.* Groups of rats, fed a stock diet, were given oral doses of aminopterin. The animals were sacrificed at frequent intervals, autopsied, and the body tissues

examined microscopically. Besides control groups, animals on a folic acid-free diet and aminopterin, and animals on water-free or starvation diets were similarly studied. The outstanding abnormalities were in the bone marrow and the gastrointestinal tract. The marrow at first showed a macrocytic and megaloblastic anemia which rapidly progressed to a total aplasia. The bowel changes were characterized by striking cellular degeneration, atrophy with cyst formation, and finally by acute ulceration and hemorrhage. The sequences of the above events were observed at frequent intervals.

### *Discussion*

(Dr. Sidney Farber, Boston, Mass.) I want to congratulate the authors on these very interesting studies. The work of our own group in the past 2 years is in essential agreement with these findings. It is worth while mentioning that aminopterin and other similar anti-metabolites bring about essentially the same effects in mice, rats, dogs with leukemia, the normal dog, the man with leukemia, and the man without leukemia. I have great doubt as to whether these effects are produced as a simple folic acid deficiency. There is a growing body of evidence to show that these substances, while producing an anti-folic acid effect, act very much further than a simple antagonism. The effect on the bone marrow is of particular interest. This effect is not limited to aminopterin. Other compounds that are in the same group bring about equal changes. All of these amino-substances seem to have an effect on a wide variety of unrelated neoplasms, both in mice and in men.

(Dr. Woll) In our experience with the chick it was possible to reverse the morphologic change, that is, in consecutive animals, by giving them large doses of folic acid. We were not able to reverse the effects with folic acid in the rat. In this case my impression is that the alterations have gone beyond the stage where reversal was possible. We are apparently dealing with a deficiency which went on to the stage where it could not be reversed. The aplasia in the bone marrow was so severe that it was hard to see how the marrow could ever regain its normal activity.

THE TRANSPLANTATION OF EXPERIMENTALLY INDUCED BRAIN TUMORS. H. M. Zimmerman and (by invitation) Nelicia Maier, New York, N.Y.

*Abstract.* All the usual varieties of gliogenous tumors have been produced in several inbred strains of mice by the implantation of pellets of chemical carcinogens such as methylcholanthrene. Rather frequently the neoplasms display histologic features which vary in different zones. Thus, for example, a single brain tumor may consist of astrocytic, spongioblastic, and oligodendrocytic parts. Homologous subcutaneous transplantation of such "mixed" tumors has made it possible to separate them into their component parts and to establish "pure" lines of these neoplasms. Continued subtransplantation of the established lines has yielded samples of astrocytoma, ependymoma, oligodendroglioma, and polar spongioblastoma. The different tumors have become behavioristically stabilized in from 8 to 14 transplant generations so that the rate of growth of each is uniform and predictable. The constancy of behavior is paralleled by the microscopic appearance. In the case of glioblastoma multiforme, which is a heterogeneous neoplasm composed of many different cell types, it has proved possible to derive several "pure" tumors by the method of serial subcutaneous transplantation. From such a single tumor there have been derived an ependymoma, an astrocytoma, and an oligodendroglioma.

For future work with homogeneous strains of gliogenous neoplasms, the tumors developed in this laboratory will be of considerable value. Among other studies they will make possible those on the chemical composition and metabolism of brain tumors.

*Discussion*

(Dr. Russell L. Holman, New Orleans, La.) I infer that the tumors did not extend outside the central nervous system. Yet they apparently will grow outside the central nervous system. I wonder if Dr. Zimmerman would care to comment on this point.

(Dr. Zimmerman) We have undertaken as part of this study another one to determine just what Dr. Holman inquired about, namely, why neoplasms which have the ability to grow outside the nervous system—and we have grown these tumors in the anterior chamber of the eye, intraperitoneally, intrathoracically, and subcutaneously—do not metastasize spontaneously. This is also true of human neoplasms. The reason, I believe, is that none of these gliogenous neoplasms have the ability to invade blood vessels and therefore cannot get out of the cranial cavity. When we transplant them outside the cranial cavity they grow perfectly well. Inasmuch as the peripheral lymphatic system has no direct connection with the perivascular spaces of Virchow-Robin, there is no connection between the lymphatics of the intracranial and extracranial portions of the body, and so these tumors have no lymphatic pathway by which to get out of the brain. But within the cranium they will invade the leptomeninges, which are mesodermal tissues, and will grow upon the dura, which is mesodermal.

FACTORS AFFECTING THE DISTRIBUTION OF TUMOR METASTASES. Richard B. Eisenberg (by invitation), Erie, Pa.

*Abstract.* The concept that certain tissues afford an unsuitable "soil" for the growth of embolizing tumor cells has been presented repeatedly in the literature. For example, the failure of tumors to metastasize to the skeletal musculature has been attributed to physical and chemical factors unfavorable to the survival of embolizing cancer cells. Another possible explanation is that metastases are absent because cancer cells, owing to their mode of metastasis, rarely reach the skeletal muscle. The experiments to be reported were designed to determine the distribution of tumors in the body when tumor cell suspensions are artificially introduced into the arterial circulation. If the cells are capable of setting up growths in skeletal muscle under these conditions, it would appear likely that the "unfavorable soil" concept is erroneous and that absence of metastases in a part can be explained by failure of embolizing cells to reach the part. The tumor used for these experiments was a squamous cell carcinoma in rabbits (the V-2 carcinoma derived from the Shope virus papilloma). This tumor, maintained by serial transfer in domestic rabbits, metastasizes regularly to the adjacent lymph nodes, occasionally to the lungs, rarely elsewhere. This behavior is similar to that of squamous cell carcinomas in man. However, when in the present experiments suspensions of cells from this tumor were inoculated into the left ventricle, multiple metastases appeared throughout the animal, in the parenchymatous organs, skin, bone, central nervous system, and muscle. Cells injected into the femoral artery produced massive infiltration of the leg muscles with solid neoplastic growth. Injection of the femoral vein and of the right ventricle produced massive pulmonary metastases with no tumors elsewhere, indicating inability of the cells to pass the lungs and failure of the cells from the developing pulmonary tumors to gain entrance to the systemic arterial circulation. From these experiments it is concluded that absence of metastases in an organ is probably due to failure of the cells to reach the part, rather than to an unfavorable "soil" afforded by the part.

*Discussion*

(Dr. Shields Warren, Boston, Mass.) This is a very useful type of study. There is evidence that striated muscle forms a good substrate for the growth of a va-

riety of malignant tumors. Take carcinoma of the tongue, for example, which grows very rapidly into the muscle. I think there is one other point to be weighed, however. If one is using an emulsion of sieved cells, you will find frequently that these are not single cells, but are clusters of cells, and that if one uses single cells, as obtained by the method developed by Gates using ascitic fluid with the tumor cells growing therein, there is opportunity for the cells to pass through the coronary vessels, at least in the rat, and to reach other organs. Another point of importance is the aggregate number of cells required to establish metastasis. It is much easier for a cluster of four or five cells to establish a metastasis than it is for many times that number of single isolated cells to do so.

(Dr. Frederic Wohlwill, Hathorne, Mass.) It is also true in man that muscle tissue is not an unfavorable soil for the growing of carcinoma. When carcinoma invades a muscle locally it may grow not only between the muscle fibers, but within the muscle fibers, so muscle tissue should be a good soil for metastasis.

(Dr. Arthur Cloudman, Chicago, Ill.) Animals of certain genetic types show metastases in 100 per cent of their ovaries and kidneys in the case of one type of subcutaneously transplanted tumor, whereas the original host type gives very little evidence of metastasis to the ovaries and kidneys. There seems to be a tendency of certain tumors, such as a reticulo-endothelioma, to give a large number of metastases in some animals, and practically no metastases in other animals. I do not know how this may be explained; I think it is not explained merely on the basis of the mechanics of the circulation.

(Dr. John W. Harman, Madison, Wis.) There are several interesting points raised by this paper concerning the possibility of what I would call barriers to the spread of tumor. This possibility is not new, but was suggested by Martin Schmidt at the turn of the century as a result of his classical studies. Dr. Paulo Dacorso and I, in a study of a series of cases of generalized spread of carcinoma, have advanced evidence of such barriers, and in this presentation we find corroboration of our findings. We observed that in instances of generalized spread, once the tumor passed beyond the lung barrier, no organ was immune to metastasis and that both the pancreas and spleen, where secondaries are believed rare, contained metastases in 50 per cent of the cases. Obliteration of the barrier in the lung was accomplished in England by Foulds by means of injections of trypan blue. We reaffirm, strengthened by this evidence of the presented paper, that there are definite barriers within the tissues, determined mainly by mechanical factors such as impaction and coagulation, and that peculiarities of soil for growth and hormonal subtleties are not required to explain the spread of tumors.

(Dr. Eisenberg) Dr. Warren mentioned that passage of tumors through a sieve results in clusters of cells as well as single cells. We are unable to state the proportion of single cells to clusters of cells in the suspensions used in these experiments, nor have we information on the relative ability of emboli of these types to form metastases.

We are unable to explain the disparity of metastases in various organs, such as kidney and ovary, in host animals and transplanted tumors, as described by Dr. Cloudman. However, it is worth emphasizing that these experiments are preliminary, and, although no evidence for the "soil" hypothesis was disclosed, it is possible that fine degrees of variation in "soils" may be disclosed by quantitative methods in which varying known numbers of cells are injected into arteries supplying various organs.

We agree that the barrier to the passage of tumor cells through the lungs is probably mechanical.



HODGKIN'S DISEASE ASSOCIATED WITH MENINGO-ENCEPHALITIS. Antonio Rottino and (by invitation) George Hoffman, New York, N.Y.

*Abstract.* This report deals with a peculiar form of meningo-encephalitis which occurred in a patient suffering from Hodgkin's disease. The patient was a white male, aged 37, with onset of Hodgkin's disease in 1942 and diagnosed as such and treated with X-ray in 1944. In May, 1945, symptoms of encephalitis developed and he died 114 days later. Gross findings at autopsy were minimal. More significant ones were the microscopic findings, which showed involvement of the Virchow-Robin spaces throughout the brain and cord. The infiltrate consisted of cells resembling epithelioid cells, occasional multinucleated giant cells, and lymphocytes. In places these were arranged in tubercles. The conclusion reached was that the lesion was neither a Hodgkin's lesion nor a tuberculous lesion. It was sarcoid in character, and it was felt that it probably represented a phenomenon arising in response to changes which had occurred in a specific biologic mechanism and which had in this case been set into motion by the Hodgkin's disease process.

#### *Discussion*

(Dr. Leo Lowbeer, Tulsa, Okla.) In view of the fact that, as Forbus and his group have shown, certain types of inflammation due to brucella morphologically resemble Hodgkin's disease, I should like to ask whether an attempt was made to culture the meningeal exudate for brucella, and whether anything is known about agglutination titer and skin allergy for brucella in this patient. The morphologic character of the meningeal granuloma as demonstrated in the slides would be compatible with that found in brucellosis. Cases of brucellic meningo-encephalitis with autopsy findings and microscopic examinations have been reported in the literature, by Hansmann and Schenken, Cull, Baggenstoss and Merritt, Scheidegger and Stern, Black, Noto, and Bergmarck, among others.

(Dr. Joseph E. Pritchard, Montreal, Canada) I would like to ask if syphilis was ruled out to Dr. Rottino's satisfaction.

(Dr. Rottino) In reply to the last question, there was no evidence of syphilis serologically. At autopsy no syphilitic lesions were found in the aorta or elsewhere.

In answer to the first question, brucellosis was not considered clinically. The picture clinically was that of encephalitis and attention was focused on it and on Hodgkin's disease. The usual bacteriologic studies were done and no organisms grown. No organisms were grown from the spinal fluid or demonstrated in the tissue sections.

HYPERSPLENISM IN MALIGNANT DISEASE—A NEW CONCEPT. Oscar B. Hunter, Jr., Washington, D.C.

*Abstract.* Observations of a case of lymphosarcoma treated with nitrogen mustard 2 years ago revealed a large spleen and the presence of a hyperplastic bone marrow. Laboratory studies during life revealed a rapid destruction of the transfused red cells and a consistently low white cell count. Post-mortem findings revealed almost complete absence of lymphosarcoma and the anatomic diagnosis was that of hypersplenism with severe anemia and leukopenia.

These findings have led to a laboratory study and correlation with post-mortem findings for a number of patients with various types of leukemia and lymphosarcoma. The essential findings are:

1. Splenomegaly due to leukemia or lymphosarcoma.
2. Hyperplasia of the normal elements of the bone marrow.
3. Exaggerated red cell destruction and/or diminution of the leukocytes and/or thrombocytes beyond what would be expected with the bone marrow findings.

4. Surgical and post-mortem studies of injected specimens reveal large dilated sinusoids lined by giant phagocytic cells containing nuclear debris and hemosiderin pigment.

5. Clinical follow-up of such patients has shown improvement following splenectomy.

The conclusions drawn from these findings are that in cases showing evidence of hypersplenism, even though due to a malignant disease, improvement can be expected from removal of the overly-phagocytic spleen. These findings are particularly applicable in some of the cases of acute leukemia treated with aminopterin where failure has resulted because of severe anemia or thrombocytopenia.

#### *Discussion*

(Dr. Joseph E. Porter, Portland, Me.) Dr. Hunter has emphasized the destruction of transfused red cells, and he has also cited a paper recently published in which hemolysis was demonstrated in lymphosarcoma and other allied diseases. I would like to know whether he has carried out Coombs' test on any of the cases investigated.

(Dr. Hunter) I regret that for the first 2 cases the Coombs' test (anti-human globulin test) was not carried out. In the last case, that of acute leukemia in which a splenectomy was done, this test was performed and was negative. It has been our experience in a series of 18 such cases that the Coombs' test is only occasionally positive and in the 2 cases in which we had observed a positive test the test has been open to considerable question. This particular state of hypersplenism I believe is not necessarily associated with hemolysins and in the cases observed we believe that splenic phagocytosis is the greater factor. This is well illustrated in the second case, that of a chronic lymphatic leukemia, which showed marked elevation of the red cell count and decrease in the required frequency of transfusions after splenectomy had been performed. Furthermore, platelet counts and white cell counts, when not influenced by medication as evidenced by the cases of lymphosarcoma, show a rise in both elements after splenectomy. From our observations of these cases we feel very definitely that the macrophages lining the splenic sinusoids have a great deal to do with the deficiency of the cellular elements in the peripheral blood.

STUDIES ON THE STRUCTURE OF ELASTIC TISSUE WITH THE ELECTRON MICROSCOPE. Jerome Gross (by invitation), Boston, Mass.

*Abstract.* Examination with the electron microscope of fragmented elastic tissue obtained from the aortas of man, rabbit and rat, bovine ligamentum nuchae, and fish swim-bladder revealed morphologically characteristic fibers differing markedly from collagen. Tryptic digestion of elastic tissue from all sources studied released large numbers of filaments, polydisperse in length, and fairly constant in diameter. A large proportion of these filaments appeared to be tightly coiled helixes composed of two intertwining threads. The distance between coils ranged between 500 and 650 Å in 100 measured filaments. The individual threads measured about 75 Å in diameter. pH was demonstrated to profoundly influence the physical state of the filaments.

These studies demonstrate the presence of two chemically and morphologically distinct components in elastic fibers. The relationship between structure and elasticity will be discussed.

#### *Discussion*

(Dr. Ernest W. Goodpasture, Nashville, Tenn.) I cannot forego the opportunity to express my personal appreciation to Dr. Gross for presenting this paper. Of course these newer methods are destined to play an enormous rôle in the fu-

ture in studies of morphology, and particularly morphology of the cell, in which we are all interested.

(Dr. H. Edward MacMahon, Boston, Mass.) I would like to ask Dr. Gross if he believes, from his studies, that collagen could be transformed into elastic tissue or if elastica could be transformed into collagen. The pictures that he has shown us of elastic fibrils as seen with the aid of an electron microscope are so completely different from those of collagen that it would not seem possible that these could be structurally interchangeable.

(Dr. Gross) Firstly, from amino acid analysis of decollagenated elastic tissue, one can see that the chemical pattern of elastin is very different from that of collagen. Elastin has somewhere near 90 per cent non-polar amino acids, whereas collagen has somewhere near 50 per cent, which would make a large difference in the organizational character and reactivity of the material; and secondly, from the fact that with the electron microscope you can see collagen fibrils passing into elastic fibers and yet the structure of the two is so different, you can imagine that on a histologic level you were seeing transitions of collagen into elastin, whereas this would actually be a passage of collagen bundles into the substance of an elastic fiber.

TISSUE RESPONSES TO PHYSICAL FORCES. IV. THE FIBROGENIC POTENTIAL OF FOREIGN PARTICLES IN TISSUE. Silas M. Evans (by invitation), Walter Zeit (by invitation), and Joseph J. Kuzma, Milwaukee, Wis.

*Abstract.* A variety of disease states are characterized by the development of fibrous tissue at the site of involvement. Oftentimes, these fibrous tissue reactions may be observed to develop in magnitude far beyond the apparent need of the situation. This apparent superfluity of fibrous tissue reactions is more striking as one considers that reactions of this type cannot be demonstrated to possess filtering or neutralizing properties to qualify them as defensive against solutions, toxins, poisons, or antigens.

The objections to the current solubility theory of silicosis are well known. Probably the most important objections appear to be that: (1) Some benign silicates provide the same ions in equal or greater concentration to the surrounding fluids as does quartz; (2) fibrous tissue is no better barrier against substances in solution than the tissue it replaced; and (3) it has never been shown that local concentrations of solution occur.

A consideration of the physical properties of fibrosive dusts reveals that, where data are established, fibrosive dusts belong to crystal classes which possess no point of molecular symmetry; whereas, benign dusts are of crystal classes with symmetric molecular structure or are non-crystalline (amorphous). Crystals which have no point of central symmetry will possess, if other conditions are proper, a property known as piezo-electricity. A piezo-electric crystal is one which is capable of transferring energy states in either direction between motion and electricity. When such a crystal is compressed mechanically, it induces an electric current; and, conversely, when it is placed in an electric current, it becomes distorted in shape. To illustrate, in phonographic reproduction, pressures induced upon a needle by the record groove are transformed through a piezo-electric crystal to the current which activates the speaker. The importance of the property of piezo-electricity in the biologic action of dusts has been explored in several ways. Rock quartz was physically reoriented to several different levels without chemical modification, and the fibrosiveness of the respective dust particles was observed. Previously untested non-siliceous ores were selected for their physical properties and fibrosiveness was correlated accordingly. The effects of physically induced energy releases within tissue were also observed.

Collagenous fibrous tissue is recognized to possess excellent tensile strength. In addition, preliminary study has indicated that this tissue, by comparison to other tissues, is highly non-conductive to heat and electricity. These physical properties qualify fibrous tissue as the best substance the body has to offer as a barrier or insulator against free energy in the form of motion, heat, or electricity. In view of these simple properties of fibrous tissue, it becomes imperative to reconsider the fibrogenic injury mechanism in those disease states hallmarked by active fibrous tissue proliferation. Such reactions would be functionally reconcilable if the injury could be demonstrated to be a release of abnormal energy within the affected part. In this case, energy in a form and magnitude sufficient to damage parenchymal cells would be localized and insulated against by the best qualified reactive tissue available to the host. The fibrogenic stimulus might then be considered to be energetic rather than material.

The fibrogenic potential of dusts is correlated with the piezo-electric activity of the dust.

#### *Discussion*

(Dr. H. Edward MacMahon, Boston, Mass.) Dr. Evans has just shown us that there is comparatively little response on the part of the body to diffuse amorphous quartz. I would now like to ask Dr. Evans if he believes it might be possible for the diffuse form of the quartz to recrystallize in the body and act as a strong fibrogenic agent. If crystallization can occur outside the body, there seems little reason why it should not take place within. I am thinking particularly of the disease, chronic beryllium pneumonitis. In this there may be a very long latent period between exposure and the disease process. Could an alteration in the physical state of the beryllium during this latent period account for the peculiar granulomatous changes in the lungs that are found in this process?

(Dr. Alan R. Moritz, Boston, Mass.) I think this is an extraordinarily interesting communication regarding a novel mechanism of physical injury. I was under the impression that adipose tissue was a poorer conductor of both heat and electricity than fibrous connective tissue. If that is the case and if the body were mobilizing its best defenses against electric or thermal injury, I should think it would lay down an adipose layer around the crystals rather than a fibrous one.

(Dr. Evans) In answer to Dr. MacMahon, vitrified silica can and indeed does revert to a crystal stage in many situations. The vitrified state is a thermodynamically unstable "high energy" state which is existent because of its high viscosity which resists molecular reorientation. Glass cutters will cut fresh glass at their own risk but refuse responsibility when asked to cut old glass. They know that with time, orientation and cleavage lines occur. We believe this mechanism to be one of the possible explanations for the fibrosiveness of amorphous materials.

In reply to Dr. Moritz, I can find very little on the physical properties of various tissues in the body. I think the work we have done with actual implants and measuring of resistances against alternating current has indicated to us that fibrous tissue is somewhat better than adipose tissue, which is paralleled by the fact that the body employs fibrous tissue as an insulating material where electropotential occurs physiologically as seen in the neurilemma.

PULMONARY LESIONS INDUCED IN WISTAR RATS BY URETHANE. F. K. Mostofi (by invitation) and C. D. Larsen (by invitation), Washington, D.C., and Bethesda, Md.

*Abstract.* Sixty Wistar rats, 5 to 8 weeks of age, about equally divided as to sex, were given single weekly intraperitoneal injections of a 10 per cent solution of urethane for 13 weeks (0.01 cc. per 1 gm. of body weight). At autopsy, 12 to 15 months later, more than one-third of the animals showed single or multiple pul-

monary lesions. The lesions were located peripherally, often subpleurally, were not elevated above the general surface level, and showed no relation to bronchi. Each consisted of a localized focus comprising several adjacent alveoli showing proliferation of alveolar cells in the stroma and within the alveoli. In 4 cases the alveolar lumina were virtually completely obliterated by a papillary cellular proliferation. There was no relation between this process and the bronchiectasis which is commonly seen in rats. The lesion was not found in control and other stock rats.

QUANTITATIVE STUDIES ON THE COLLATERAL CIRCULATION OF THE LUNG AFTER LIGATION OF THE PULMONARY ARTERY. William E. Bloomer and William Harrison (by invitation), Averill A. Liebow, and (by invitation) Milton R. Hales and Gustaf E. Lindskog, New Haven, Conn.

*Abstract.* By bronchspirometry and an application of the Fick principle to data obtained by bronchspirometry and analysis of the systemic arterial blood, the collateral circulation of the lung after ligation of the pulmonary artery was demonstrated to increase enormously. Most of the increase occurs within the first 2 weeks, but it is slowly progressive for at least 18 months. At the end of this time the collateral flow is approximately 1 l./m.<sup>2</sup>/min.

The extent of the collateral bed demonstrated anatomically in vinylite corrosion casts and in histologic sections appears to be compatible with the magnitude of the flow as determined in the physiologic experiments. The bronchial arteries associated with the terminal bronchi now have a larger total area in cross section than the pulmonary arteries at the same level. This, together with the absence of morphologic changes in the walls of the alveoli, suggests that the peripheral resistance to blood flow in the lungs remains low.

The pulmonary artery does not become thrombosed distally, but comes into communication with the bronchial arteries through pre-capillary anastomoses that come to exceed 50  $\mu$  in diameter. Thus the pulmonary artery becomes a huge diverticulum of the bronchial arterial circulation and may, in part, absorb the impact of the systemic arterial pressure before the capillaries of the alveoli are reached. Under the conditions of the present experiments most of the blood entering the lung via the bronchial arteries is returned directly to the left auricle through the pulmonary veins. Thus the collateral circulation, which may exceed in volume one-third the output of the right ventricle, is a burden entirely upon the left side of the heart.

#### *Discussion*

(Dr. Jesse E. Edwards, Rochester, Minn.) This paper is of considerable interest. In a consideration of the differential pressures which must exist between the bronchial arteries and the pulmonary arteries under normal conditions and under conditions in which the bronchial arteries are dilated, the human being and some of the experimental material are not proper subjects for the measurement of such differences in pressures. It would seem that we might possibly have a means of doing this by cannulating the left pulmonary artery beyond the point of ligation and also the right pulmonary artery, to determine whether there exists a differential pressure between the two lungs. I should like to ask Dr. Liebow whether he has any evidence concerning the pressure in the lung which is receiving its blood by way of the normal pulmonary circuit on the one hand, and the lung which is receiving its circulation through the collateral system derived from the aorta on the other.

(Dr. Liebow) In answer to Dr. Edwards' question, we had not intended to go into this problem at this time because we do not have a great deal of evidence on the point in regard to the dogs. In the case of chronic, severe, unilateral bron-

chiectasis in man, however, before any operative procedure is done the two pulmonary arteries can with good fortune be entered separately by means of catheters. When that is done the pressure on the diseased side, as determined by Hamilton manometry, is considerably in excess of the pressure in the relatively normal side. This suggests that blood may be flowing backwards in the pulmonary artery of the diseased side toward the hilum of the lung. That is rather easy to understand because of the large size of the anastomoses between the two systems and the fact that the blood in the expanded bronchial arteries is under high pressure.

One additional proof is necessary—to determine whether the blood in the pulmonary artery on the side of disease has the same characteristics as the blood in the femoral artery. That is, indeed, the case.

THE PATHOGENESIS OF EXPERIMENTAL FAT EMBOLISM. John W. Harman, and Florian J. Ragatz (by invitation), Madison, Wis.

*Abstract.* Fat embolism was produced in rabbits by the intravenous injection of homologous liquid fat, obtained by alcohol extraction of perinephric fat. The volume of fat injected was proportional to body weight and the lethality of the dose thereby determined. It was found that the mortality rates for 0.45, 0.55, 0.75, and 0.90 ml. of fat per kg. of body weight are, respectively, 16.5, 50, 66, and 100 per cent. Dosage *per se* has a decided effect upon the mortality rate, whereas rate of injection and repetition have slight effect. With a constant dose the effect of other factors upon mortality can be examined.

A series of rabbits was studied for the influence of trauma on the mortality due to fat. Trauma was instituted by the application of a tourniquet to one thigh so as to occlude the blood supply completely for 4 hours. An hour after release of the tourniquet fat was injected, 0.45 ml. per kg. intravenously. The mortality rate was 41 per cent in contrast to 16.6 per cent for the fat-injected controls and 0 per cent for the 60 traumatized controls. Because of the considerable fluid loss into the traumatized limb, another series of rabbits was dehydrated by water deprivation and injection of hypertonic glucose. These suffered considerable weight loss and some hemoconcentration. The injection of 0.45 ml. per kg. of fat produced a 50 per cent mortality with extremely rapid death as compared with the controls. This suggests that dehydration may be a factor in the deaths due to trauma combined with embolism.

Both gross and histologic studies revealed the principal changes to be in the lungs, which were very edematous, studded with minute areas of hemorrhage, and infiltrated with polymorphonuclear leukocytes. Animals with the most extensive pulmonary lesions often had a widespread centrilobular coagulation necrosis of the liver cords. Ascites and pleural effusion were also encountered. These lesions suggested anoxic anoxia as the chief disturbance. To test this, a series of rabbits injected with 0.55 ml. per kg. of fat were maintained in an atmosphere of 80 per cent oxygen for a period exceeding the survival period of untreated fat-injected controls. This therapy reduced the mortality from 50 per cent to nil, with slight alteration of the pulmonary lesion, but obviation of hepatic changes. The pulmonary alteration was qualitatively similar to the controls, though less extensive. In a small additional group of rabbits injection of paraffin oil was characterized by a higher mortality as compared with homologous fat. The action appeared mechanical, however, because the lungs contained no lesions or edema, such as was seen with the fat injections.

It may be concluded, therefore, that fat in higher doses may cause death by mechanical obstruction, and in low doses damages the pulmonary intimate vasculature. The damage results in stasis, edema, and anoxic anoxia, which may cause

death. This may be prevented by adequate oxygen therapy, although the development of the lesion is unaffected. On the other hand, both dehydration and tourniquet shock augment the mortality significantly. It is suggested that the trauma acts by dehydration associated with fluid loss into the traumatized area.

### Discussion

(Dr. O. J. Pollak, Quincy, Mass.) I would like to ask two questions: 1, as to the size of the fat globules injected, 2, as to the presence of fat in cells of the subintimal layer and the fat stains used.

(Dr. Harman) The size of the fat globules may be determined roughly by watching the fluid break up as it enters the blood stream, but the injection was at such a rate that we could not observe their exact size. We made no attempt to produce an emulsion or suspension of uniform particles.

As to the stains for fat, we did extensive fat stains on all of the tissues, and particularly stained the tissues in which necrosis occurred. We do not believe that the use of the Nile sulfate stain is applicable or reliable. We were especially interested in the fat in the liver and tried to exclude the possibility of fat emboli passing into the peripheral portion of the lobule and producing central ischemic necrosis. We did not find either paraffin or neutral fat under the circumstances. The subintimal cells of pulmonary arteries, so far as we could determine by means of sudan III stains, did not contain demonstrable fat. We presume that the swelling of these cells, therefore, is due to the imbibition of fluid other than fat.

### BONE CHANGES OBSERVED FOLLOWING INTRAVENOUS INJECTIONS OF BERYLLIUM.

A. M. Cloudman, Dorothy Vining, S. Barkulis, and J. J. Nickson (all by invitation), Chicago, Ill.

*Abstract.* It was reported several years ago that intravenous injections of insoluble beryllium compounds can produce bone tumors in rabbits (L. U. Gardner and H. F. Heslington, *Federation Proc.*, 1946, 5, 221). The work reported here was started to confirm the above and to extend data to include mice.

The animals were arranged in groups called I, II, III, and IV, which received zinc beryllium silicate, zinc silicate, beryllium oxide, and no treatment, respectively. The rabbits were injected through the ear veins and the mice received tail vein injections. Semi-weekly injections were made until all animals had received a series of 20 to 22 injections. The group I rabbits each received a total of about 550 mg. of Zn and 17 mg. of Be; the mice in this group each received about 8.36 mg. of Zn and 0.264 mg. of Be. In group II the total dosage to each rabbit was about 390 mg. of Zn while each mouse received about 2.8 mg. of Zn. The beryllium oxide used in group III was substantially 100 per cent BeO by spectrographic standards. The rabbits in group III received a total of about 390 mg. each and the mice received 1.54 mg. each.

In the rabbits bone changes were followed by means of periodic X-ray films of the entire skeleton. Terminal X-ray films were taken of the mice. The rabbit films showed that only group I animals, or those receiving  $\text{ZnBeSiO}_3$ , showed bone changes. Irregular thickenings of the cortices of the long bones were observed as early as 94 days after injections were started. These changes were most evident in the humerus, femur, and tibia. The bones of the pelvis and skull also showed some increase in density. This thickening did not proceed at the same rate in all rabbits of group I, yet 4 of 5 animals that lived over 1 year from the start of the injections have developed bone tumors. All but one of these bone tumors showed gross evidence of metastases. Group I mice also showed some bone tumors that were malignant. This is not true to date of the mice in the other groups.

*Discussion*

(Dr. Russell L. Holman, New Orleans, La.) I should like to ask what form of beryllium was used, what compounds or "trade names" we should be on the look-out for, and if Dr. Cloudman knows any compounds other than beryllium which might cause such bone changes.

(Dr. Frank Dutra, Cincinnati, Ohio) I would like to state that pure beryllium oxide, administered intravenously, has been found capable of producing tumors in rabbits, in the same way that these tumors occurred in the animals discussed here. The carcinogenic activity does not depend upon the zinc and silica of the substance. We were able to induce almost exactly the same number of tumors using pure beryllium oxide as with zinc beryllium silicate.

I would like to comment on the possibility of tumors due to beryllium occurring in man. We have been interested in this, and we are convinced that no such tumors have occurred to date. The amount of beryllium administered to these animals, per unit of body weight, is far greater than any human is likely to have gotten in industry. The amounts recovered from the tissues of our rabbits with tumors far exceed the quantities recovered from any employee who died of either acute or chronic pulmonary berylliosis. It may be that the route of administration is highly important in determining carcinogenic activity; I would be delighted to have Dr. Cloudman's comments on this.

(Dr. Cloudman) I do not know of any non-radioactive elements other than beryllium that produce bone tumors. Some of you may know of some, but I do not.

As for the hazards, these rabbits got 17 mg. of beryllium element, that is, they got about a gram of the zinc beryllium silicate, and many of the rabbits that got the tumors first had less than that. Just how much danger there is of getting bone tumors in industry I do not know. This is the result of intravenous injection, and yet I think that anything that can result from beryllium should be investigated and reported upon. I know that some investigators have produced bone tumors with beryllium oxide, and we were unable to do that, although I must say that our animals receiving beryllium oxide did not survive as long as I would like to have had them survive. The minority survived a year, but I had X-rays of the bones that showed no changes in the terminal state.

**HISTORADIOGRAPHY—A STUDY OF CHEMICAL ELEMENTS IN HISTOLOGIC SECTIONS BY MEANS OF X-RAY ABSORPTION.** Leonidas Marinelli (by invitation), Gaetano Ferlazzo (by invitation), Patrick J. Fitzgerald, and Frank W. Foote, New York, N.Y.

*Abstract.* Radiography is based on the fact that X-rays are absorbed by matter and that the amount of absorption is dependent upon the wave-length of the X-rays as well as on the density and atomic number of the absorbing material. Industry has utilized these facts in the soft X-ray region to investigate the fine structure of metals and alloys. Soft X-rays have been applied to biologic studies such as insect anatomy, and the structure of plant and animal life. Lamarque and others have used this technic with histologic sections and obtained differences in X-ray absorption by various substances in skin, thyroid, cartilage, and blood vessels.

It is well known that the absorption of X-rays by a chemical element will change abruptly at the wave length which is specific for the chemical element in question. Such discontinuity (absorption edge) permits the identification of an element in the presence of others, if proper technics are employed. A few elements have been identified and measured in histologic sections by Engström (calcium in blood



vessels, calcium and phosphorus in dentine of teeth, and sulfur in skin). The latter states that extremely small amounts of these elements,  $10^{-10}$  gm. or less, can be determined in a tissue area of about  $100 \mu^2$  with an estimated analytic error of 10 per cent. The physics department at Memorial Hospital has developed a technic whereby routine paraffin-embedded sections of surgical specimens of human or animal thyroid gland,  $10 \mu$  thick, can be studied for their iodine content keeping the tissue intact so that the histologic detail can be correlated with the characteristic absorption effect. The paraffin section is floated onto a photographic plate, dried, and with the tissue overlying the photographic emulsion, both are exposed to an X-ray beam that is preferentially absorbed by the element iodine. Areas that contain colloid in normal thyroid glands absorb these X-rays and no reduction of the underlying photographic emulsion occurs. All areas other than colloid allow the passage of X-rays which on reaching the photographic emulsion reduce it. When the photographic plate is processed, the areas of absorption (containing colloid and presumably iodine) are light. The non-absorbing areas are dark. Correlation of the histologic detail with light absorption can be made. Since most of the X-rays from our tube are of a wave length close to the L absorption edge of iodine ( $2.7 \text{ \AA}$ ), we believe that we have demonstrated iodine in the sections presented.

We have studied about 600 historadiograms in the development of the technic. Normal human thyroid showed absorption in the colloid of the follicles. Follicles differed considerably in amounts of absorption and even adjacent follicles varied widely. Papillary adenocarcinoma of the thyroid revealed no absorption in papillary areas but a few intact thyroid follicles in the same section showed absorption. Follicular and alveolar adenocarcinoma demonstrated absorption of varying degrees where colloid was present.

Theoretically, it is possible to apply the method of historadiography to the localization of all chemical elements other than hydrogen; however, many technical difficulties are anticipated in the identification of elements of very low atomic number.

#### ANTIGEN TRACER STUDIES AND HISTOLOGIC OBSERVATIONS IN ANAPHYLACTIC SHOCK IN THE GUINEA-PIG (PART II). Frank Dixon (by invitation) and Shields Warren, Boston, Mass.

*Abstract.* Crystalline egg albumen combined with a radioactive isotope of iodine,  $I^{131}$ , was used as a labelled antigen in the study of anaphylaxis in guinea-pigs. Three groups of animals were studied: the first, sensitized with non-radioactive, iodinated albumen and shocked with radioactive, iodinated albumen; the second, non-sensitized controls, injected with a single dose of radioactive albumen; the third, sensitized and shocked with fibrinogen and with an injection of radioactive albumen made simultaneously with the shocking dose of fibrinogen. The radioactive albumen was traced in the tissues of these animals by means of Geiger counts and autoradiograms.

The most significant difference between the antigen distribution in shocked animals (group I) and non-sensitized controls (group II) was in the lungs. Shocked lungs contained more than one and one-half times as much antigen as lungs of controls. Autoradiograms showed the increased amount of antigen to be in the edematous collagenous tissue of the bronchial walls and of the adventitia of pulmonary vessels. This collagenous tissue in the controls was not edematous and contained no antigen.

To determine whether this localization of antigen about the bronchi and pulmonary vessels in anaphylaxis was a function of the specific antigen or the result of a non-specific leakage of protein through permeable vessels, fibrinogen was used

as the sensitizing and shocking antigen in the third group of guinea-pigs, and labelled albumen was given simultaneously with the shock dose. The labelled albumen content in the tissues of these animals closely approximated that in the non-sensitized controls. The amount of antigen in the lungs was not increased over that in the controls, and only a small amount could be demonstrated in the bronchial and vascular walls. This suggests that the localization of the antigen in the edematous collagenous tissue of the bronchial and vascular walls in anaphylactic shock may be a specific function of the causal antigen.

The mechanism of bronchial obstruction in albumen anaphylaxis which terminated fatally in 2 minutes was somewhat different from that observed in the previously reported globulin anaphylaxis which terminated fatally in from 5 to 8 minutes. The rapidly fatal obstruction in albumen shock resulted from a combination of smooth muscle contraction seen around a few of the bronchi and edema in most of the bronchial walls. In the more prolonged globulin shock, smooth muscle contraction occurring in the first 1 to 3 minutes had disappeared by the time of death, and the only significant finding was extensive edema in the walls of almost all of the medium and large bronchi. It thus seems likely that smooth muscle spasm is a transitory phenomenon in anaphylaxis and is most important during the first few minutes of shock. Edema, on the other hand, apparently increases, becoming a progressively greater factor in bronchial obstruction in prolonged shock.

#### *Discussion*

(Dr. Jerome Gross, Boston, Mass.) Would this radioactive antigen tracer technique demonstrate which components of connective tissue are involved in this process?

(Dr. Dixon) This method is not as yet sufficiently refined to localize radioactive materials of this type to individual cells or individual fibers. About the best we can do is to localize it to individual areas, such as the peripheral parts of the bronchial wall.

#### **SERIAL BIOPSY OF THE TESTIS: OBSERVATIONS IN ENDOCRINE DYSFUNCTION AND STERILITY. Ronald C. Sniffen (by invitation), Boston, Mass.**

*Abstract.* Testicular biopsy was employed in patients with various types of hypogonadism as an aid to diagnosis and the evaluation of therapeutic measures. At the close of the series over 300 men and boys had been studied. The patients fell into two large groups: (a) apparently sterile individuals without detectable endocrine dysfunction, but with oligospermia or azoospermia; and (b) those with obvious endocrine disorders.

In the first group approximately half the patients showed abnormalities of spermatogenesis in the form of either hypospermatogenesis, arrest of spermatogenesis, or complete absence of germ cells. The other elements of the tubules were not affected. The other half of the group showed an essentially normal gland, and at operation were found to have congenital or acquired defects in the excretory duct system. In the absence of endocrine dysfunction, definite abnormalities in the interstitial cells were not observed. Hormone therapy had no appreciable effect on spermatogenesis in this group.

In this presentation only patients with the following abnormal endocrine manifestations are considered: (a) eunuchoidism with low follicle-stimulating hormone (FSH) values, (b) patients with hypophyseal injury, and (c) precocious boys. It was found that in patients with eunuchoidism with low FSH values and an immature testis, testosterone produced tubular maturation and stimulated spermatocytogenesis, but had no noticeable effect on the interstitial tissue. APL, on the other hand, led to Leydig cell development and tubular maturation with stimula-

tion of spermatocytogenesis. Both forms of therapy resulted in the return to normal of a tunica propria previously showing collagenous thickening.

In the presence of an intracranial lesion that damaged the hypophysis the testicular picture varied with the age of onset and the severity of the damage. If the onset occurred before puberty and resulted in hypopituitarism, the testis remained in an immature state. If the hypophysis was injured after puberty, the testis showed regressive changes that seemed to be characteristic of hypophyseal suppression. The features of this regression consisted of hypospermatogenesis and finally aspermatogenesis, and accumulation of fatty substances in the Sertoli cell cytoplasm, collagen and elastic tissue deposition in the tunica propria, and gradual atrophy of the Leydig cells. In a series of testes from men with carcinoma of the prostate treated with stilbestrol for varying intervals before orchiectomy, the tubules and Leydig cells followed the same path of regression as in organic hypophyseal suppression.

Precocity in children is of two main types: (a) "central" precocity, where the stimulus presumably arises in the hypothalamus or hypophysis, and (b) "adrenal" precocity due to hyperplasia or carcinoma of the adrenal cortex. Testicular biopsy in a small group of children indicated that in "central" precocity both tubules and Leydig cells are stimulated, while in "adrenal" precocity the tubules mature but there is no stimulation of spermatogenesis or Leydig cell formation.

#### TISSUE REACTIONS TO SUBCUTANEOUS IMPLANTS OF THERMALLY DENATURED PORCINE SKIN. Richard Ford, Boston, Mass.

*Abstract.* The observation that severe human burns are subject to unpredictable rates and types of reparative processes has led to the following study of the denaturation of skin by controlled *in vitro* heating. Using a controlled-temperature, circulating bath of water or mineral oil of  $\pm 0.1^\circ$  C. accuracy, segments (approximately 5 by 7, mm.) of freshly excised skin from weanling domestic hogs were heated inside thin synthetic rubber sheaths at various temperatures from  $45^\circ$  to  $125^\circ$  C. The heated skin was then implanted in subcutaneous pockets in the donor hog for periods of about 4 weeks.

An initial series heated for 10 minutes after the tissue reached the bath temperature and implanted for 25 days gave the following results: At  $40^\circ$  and  $50^\circ$  C. epidermis survived and grew upon the original dermis or upon new stroma without rete pegs. The implanted dermis proliferated marginally and merged with the fibrous tissue of the surrounding area. At  $53^\circ$  and  $54^\circ$  C. no epithelium survived and the dermis was broken up and replaced by invading strands of new fibrous tissue with some inflammatory exudate. Scavenger cells, including foreign body giant cells, were effectively removing traces of dead epithelium, hair, and cornified layers. Between  $55^\circ$  and  $90^\circ$  C. the dermis was encapsulated by a thin zone of new fibrous tissue. The dead epithelium, hair, and cornified layers were again effectively subject to scavenger cell action. At  $100^\circ$ ,  $115^\circ$ , and  $125^\circ$  C. the dermis and epidermis were surrounded by a wide zone of coarse fibrosis infiltrated by an exudate predominantly neutrophilic. The exudate had invaded the heated dermis without concomitant fibrosis and masses of leukocytes appeared to have replaced the dead dermis by lysis. The dead epithelium and appendages evoked moderate foreign body scavenger reaction.

Two additional series were implanted after being brought to the desired temperature and then immediately cooled in a room temperature water bath. Tissue temperature was recorded by wrapping the sub-dermal face of the tissue around the thermocouple tip and tying with thread. In one series, time of heating from  $50^\circ$  C. to the desired temperature and back to  $50^\circ$  C. was recorded in each case and it varied from 38 seconds for  $51^\circ$  C. to 147 seconds for  $70^\circ$  C. Epithelium

survived on the original dermis with persistent rete pegs at 50°, 51°, and 52° C. At 53° and 54° C., also, epithelium persisted but appeared only on a new flat stroma and was pale-staining, flattened, and relatively less cellular. At 53° and 54° C. the implanted dermis appeared dead in that it was non-proliferating and vascularized by capillaries surrounded by new connective tissue. From 55° through 70° C. epidermis and dermis were dead. There was slight encapsulation of the dermis without vascularization, fibrosis, or exudative reaction of the dermal mass. The epithelial detritus evoked marked scavenger cell reaction.

In these three series epidermis was killed by heat at 55° C. and dermis at a slightly lower temperature when heating was brief. As in the case of certain other forms of noxious radiant energy, heat appears to injure the dermis sooner than the epidermis. Dermis was heat-fixed above 55° C. and remained unaltered until 100° C. Above 100° C. it became subject to lysis and infiltration by inflammatory cells. Nearly lethal temperatures appear to denature the living epidermal epithelium.

### *Discussion*

(Dr. Shields Warren, Boston, Mass.) I would like to ask if there is any parallel study as to the change of the proteins of the porcine skin which might give a clue to this variation in reaction at the two temperatures you have described.

(Dr. Ford) I understand that biologists of the Massachusetts Institute of Technology have been working on this matter, but I am not familiar with this phase of their work. I feel that the problem from my own angle is still incomplete.

### HISTOPATHOGENESIS OF CARCINOMA OF THE FORESTOMACH OF MICE INDUCED BY INTRAMURAL INJECTION OF 20-METHYLCHOLANTHRENE. H. I. Firminger (by invitation) and H. L. Stewart, Bethesda, Md.

*Abstract.* An attempt was made to characterize early neoplastic changes in stratified squamous epithelium by studying the histopathogenesis of squamous cell carcinoma of the forestomach in mice. One one-hundredth cc. of an aqueous methocel suspension containing 30 mg. of methylcholanthrene per cc. was injected into the wall of the forestomach of 200 young adult mice of strains C<sub>57</sub> brown and A backcross, equally divided as to sex. They were killed on the average of 4 per week for 12 months.

Histologically, within the first 3 days there was hyperkeratosis, acanthosis, mucosal edema and irregularity of basal cells overlying the injection site. There was a reaction about the methylcholanthrene in the submucosa characterized by edema, inflammatory change, necrosis, and degeneration of collagen. Subsequently, the mucosa showed various abnormal hyperplastic and dyskeratotic changes. The basal layer thickened progressively, its cells became irregular in shape, staining, and orientation and formed small buds. In occasional foci the basal cells were lost owing to degeneration, leaving the spinous cells in direct contact with the lamina propria in that area. Several retia showed inversion of keratinization at their growing tips. Disorder of keratinization was reflected by pre-eleidin granules or single cell keratinization even in the basal layer, and by abnormality of the granular layer, parakeratosis, and "corps ronds" formation. Squamous cell carcinoma occurred first at 39 days and was frequent after 2 months. Sarcomas were infrequent and did not occur until 91 days or more after injection.

### THE INFLUENCE OF PHOSPHORUS UPON THE ABSORPTION OF IRON. T. D. Kinney and (by invitation) D. M. Hegsted and C. Fitch, Cleveland, Ohio, and Boston, Mass.

*Abstract.* It has been shown that rats fed a diet of 80 per cent corn grits and 20 per cent lard plus haliver oil absorbed and deposited in the liver large amounts

of iron when excessive amounts of ferric citrate were added to the diet. Animals fed control diets of purina dog chow containing the same amount of ferric citrate showed an insignificant increase in the liver iron. This increase in iron in the livers was not due to a redistribution of body iron following the loss of body weight, since animals fed the corn grit diet without added iron lost comparable amounts of weight and there was only a slight increase in liver iron values. Also, the liver iron in some animals which received the corn grit diet with iron for long periods was greater than the total body iron of normal rats. On the basis of these data it was concluded that the normal intestinal block to iron absorption fails under certain conditions.

Experiments were designed to determine the dietary factors primarily involved in the failure of animals fed the corn grits and lard diet to control iron absorption. From these experiments it was concluded that the excessive absorption and deposition of iron in the livers of the animals on the diet are the result of the low phosphorus content of the diet. Further, it has been shown that it is possible to obtain excessive iron deposits in the livers of animals receiving a normal diet by adding large amounts of iron salts to the diet. These experiments indicate that non-absorption can be changed by altering either the phosphorus or the iron content of the diet. Analysis of the data obtained further indicates that the absolute amount of iron and/or phosphorus in the diet, as well as the iron/phosphorus ratio, influences the amount of iron absorbed.

RENAL EXCRETION OF CHOLESTEROL. Timothy Leary, Boston, Mass.

*Abstract.* In rabbits fed cholesterol the substance, in the form of crystalline esters, may appear in the kidney in tubular epithelial cells of the distal convoluted tubules and Henle's loops, first in the intermediate zone of the cortex and pyramids. Associated with this is the accumulation of cholesterophages in the inter-tubular connective tissue. Necrosis of these phagocytes may free crystalline esters, and splitting of the esters with disappearance of the fatty acids may lead to precipitation of solid cholesterol crystals. When feeding of cholesterol is continued for long periods, massive deposits of solid cholesterol crystals in the pyramids and the resulting fibrosis may cut off nephrons and give rise to nephrosclerosis. Observations under polarized light illustrate the presence of crystalline cholesterol in the lesions.

In human kidneys the excretion of crystalline ester cholesterol with damage to renal epithelial cells is manifested in glomerulonephritis, in lipoid nephrosis, and in lower nephron nephrosis. The appearance suggests that cholesterol is normally excreted in non-visible form, perhaps as ketosteroids. Polariscopic evidence of the excretion of visible forms of cholesterol is found almost exclusively in diseased kidneys.

CRYSTALLINE ESTER CHOLESTEROL AND RENAL ADULT CORTICAL TUMORS. Timothy Leary, Boston, Mass.

*Abstract.* Adult cortical renal tumors are found usually in persons, largely men, over 40 years of age, and in kidneys that tend to be nephrosclerotic. In such kidneys are sometimes found miniature papillary tumors, singly or in numbers, whose relation to renal cell tumors is close. In kidneys with multiple tumors fresh sections under polarized light disclose that the beginning and the progression of such a tumor is linked with the focal deposit of crystalline esters of cholesterol in cells of a proximal convoluted tubule causing the cells to swell. Then the affected portion of the tubule is sealed off from the rest of the tubule and undergoes cystic dilatation. The swollen cells proliferate. Blunt papillary stems are produced which begin to branch and then to produce a complex papillary new growth. In some

tumors the crystalline ester cholesterol is suddenly cast out of the tumor cells, and the tumor may stop active growth and subside to become a simple tubular adenoma, the cells of which contain no fat-staining material or crystalline ester cholesterol. In others, growth progresses, associated with evident focal accretions of cholesterol and with evidence of the removal of crystalline ester cholesterol piecemeal in macrophages. Steps in this progression and regression are illustrated.

### *Discussion*

(Dr. Shields Warren, Boston, Mass.) It certainly would be worth while for all of us who run across these tumors to attempt in some small way to make studies of the type which Dr. Leary is carrying on. I think he has called our attention to a most interesting change, and the alteration in the cells that have been forced to depart from their normal functions to structural and perhaps organic change is of very real interest.

### READ BY TITLE

ALLERGIC GRANULOMATOSIS. Jacob Churg and Lotte Strauss (by invitation), New York, N.Y.

*Abstract.* During the past 25 years a relation between allergic states and lesions of the type seen in periarteritis nodosa has been firmly established by experimental evidence and the study of human disease. Such lesions usually are limited to the blood vessels; there are, however, reports in the literature indicating that widely dispersed extravascular lesions may occur in the connective tissues. We have observed several cases of what might be considered typical periarteritis nodosa, accompanied by prominent lesions in the extravascular connective tissue, consisting of severe collagen alteration, exudation, and histiocytic proliferation. In another group of cases there were spent vascular lesions and still active extravascular changes. In one instance lesions of the same histologic type were limited to connective tissue while the vascular system showed practically no change. All 7 cases had a history of severe allergy, particularly asthma; furthermore, they ran a febrile course and showed blood eosinophilia.

A typical extravascular lesion had the appearance of a granulomatous nodule, often large enough to be seen with the naked eye. Histologically, it consisted of an eosinophilic core in which one could discern more or less severely altered collagen fibers mixed with varying numbers of disintegrating eosinophilic leukocytes. Where the collagen fibers could still be recognized they appeared markedly swollen and eosinophilic, and in polarized light they failed to show the usual double refractility. At times eosinophilic leukocytes were so numerous that one could not be sure of an underlying collagen change. The center of a nodule was surrounded by radially arranged epithelioid cells mingled with multinucleated giant cells of Langhans' or foreign body type, and beyond that, by a layer of exudate composed of eosinophilic leukocytes together with plasma cells and macrophages. One could observe various stages of evolution in the granulomas, from active exudation to more or less complete fibrosis. Calcification of the center occurred on occasion. In some cases there was associated eosinophilic pneumonia with giant cells, as seen in Loeffler's syndrome.

CODEINE POISONING: FATAL CASE FROM ACCIDENTAL OVERDOSE. V. H. Cornell, Washington, D.C.

*Abstract.* Death from codeine is so rare that only one case, that of an asthmatic patient, is found recorded. The present case is therefore reported primarily to record it for future reference and to indicate that this drug may be lethal in large doses.

A 31-year-old male accidentally drank 1 to 2 oz. of an improperly prepared codeine cough mixture, thus receiving between 13 and 26 gr. (0.9 to 1.75 gm.) of codeine sulfate. Concentrations found on toxicologic examinations were: brain, 0.5 mg. per cent; liver, 0.9 mg. per cent; urine, 12.5 mg. per cent; blood, 1.0 mg. per cent. The patient was an asthmatic and also under phenobarbital medication, the blood concentration of this drug being determined as 0.7 mg. per cent. An area of hemorrhage in the epicardium and auricular musculature in the region of the sino-auricular node may have been the final fatal lesion and probably was due to the anoxia induced by the medication. Death occurred in 12 to 15 hours.

ENCEPHALITIS IN DIPHTHERIA. Vera B. Dolgopel and (by invitation) Selig H. Katz, New York, N.Y.

*Abstract.* The report is based on the examination of the brains of 11 patients who died from 3 to 66 days after the onset of diphtheria. True encephalitis was found in 5 cases. The lesions consisted of (1) mononuclear infiltration of the venous walls in one or several locations (medulla, basal ganglia, frontal cortex, infundibulum), and (2) microglial nodules in the medulla (nucleus ambiguus, spinal vestibular nucleus). Severe cellular degeneration was rarely encountered, but scattered shrunken and hyperchromatic cells were frequent in the medulla. In another case no cellular infiltrates were found, but a few binucleated ganglion cells were seen in the medulla; this finding suggests that an inflammatory process had been present in the central nervous system at an earlier date. No demyelination was observed in the brain tissue or in the nerve roots of these 6 cases. Myocarditis was present in 4 of the 6 cases, but apparently played no part in producing cerebral lesions, as none of them were of embolic character. Severe mental depression was observed in 1 patient, palatal paralysis was present in 2. Encephalitis in diphtheria could be the result of the action of the circulating toxin on the brain tissue and its blood vessels, or could be attributed to activation of some latent virus by the diphtherial toxin.

MALIGNANT HEMANGIOPERICYTOMA OF POPLITEAL SPACE. Robert Fienberg, Framingham, Mass.

*Abstract.* This case report concerns a 34-year-old white male with a mass of 8 months' duration behind the left knee. Biopsy revealed a malignant tumor, and a mid-thigh amputation was done. Examination of the surgical specimen showed a large lobulated mass involving the left popliteal space. The knee joint was clear. Microscopic examination revealed numerous thin-walled blood vessels surrounded massively by hyperchromatic cells with oval to round nuclei varying little in size and shape. No proliferation of endothelial cells was noted. The cells often grew in solid compact form. There was little necrosis. A reticulum stain showed a characteristic picture. There was marked invasion of the soft tissues of the popliteal space, including the striated muscle.

Since the hemangiopericytoma was first described by Stout and Murray in 1942, only a very few of the malignant variants have been described and published, so that very little is known concerning their characteristics. The slides of this specimen were examined by Drs. Fred W. Stewart and Arthur Purdy Stout, both of whom concurred in the diagnosis of malignant hemangiopericytoma.

SIMPLIFIED METHOD OF ALCOHOL ESTIMATION IN NON-DISTILLED VITAL FLUIDS. William E. B. Hall, St. Louis, Mo.

*Abstract.* With certain modifications, filtrates of protein-free blood and other materials are susceptible to colorimetric estimations of alcohol contents by the Duboscq colorimeter, preferably by the photo-electric colorimeter, without the in-

tervening steps of distillation, and particularly without the necessity of other substage titrations as presently used, as for instance, in the iodine titration method.

THE BASEMENT MEMBRANE OF THE FETAL PLACENTAL VILLUS AND ITS RELATION TO TOXIC AND PATHOLOGIC OBSTETRICAL STATES. William E. B. Hall, St. Louis, Mo.

*Abstract.* Except for a short presentation in an obscure medical publication, there appears to have been no identification or description of a basement membrane of the fetal placental villus. This structure has been identified as being histologically distinct, of a material related to, but not identical with, reticulum, and with staining qualities and characteristics similar to those of the vessels of the villi and the basement membrane of the renal glomerulus. This structure appears at approximately  $1\frac{1}{2}$  months of gestation and microscopically exhibits a pronounced affinity for calcium in certain placental degenerative states and with retention. Changes, particularly in the nature of thickening, are observed in toxic states, especially in association with intervillous thrombosis. The effect of thrombo-infarctions on the filtration function of the placental villus is reflected in the thickening and alterations of the basement membrane. This histologic and apparent functional change appears identical with that observed in the basement membrane in the various nephritides. The functional disturbance where the placental villus is so affected appears analogous to alterations in nephritic states and is expected to be accompanied by comparable toxic and hypertensive effects on the maternal and fetal organism, as well as distinct disturbances of the nutritional and excretory functions.

SQUAMOUS CELL METAPLASIA AND THE DIAGNOSIS OF LUNG CANCER. Samuel R. Haythorn, Pittsburgh, Pa.

*Abstract.* Squamous cell metaplasia of the bronchial epithelium has been found to be a relatively common lesion in the surgical and autopsy materials at the Allegheny General Hospital. It has been found in cases of bronchiectasis, abscessed lungs, organizing pneumonia, tuberculosis, and in non-cancerous portions of lungs showing squamous cell bronchogenic cancer. Squamous cell cancer has been the commonest type found in our series. A group of cases showing squamous cell metaplasia will be presented. They represent conditions in which the pathologist is frequently asked to make a differential diagnosis by the smear method. Desquamated epithelium from metaplastic areas may show prickles or cornification, and resemble squamous cancer cells closely. Where squamous cells are the only ones arousing suspicion in a smear for the diagnosis of bronchogenic cancer, a positive diagnosis should be made with reservations.

PLASMOCYTOMA OF THE THYROID. John B. Hazard and (by invitation) W. W. Schildecker, Cleveland, Ohio.

*Abstract.* Plasmocytoma of the thyroid is a rare disease, only one case having been reported during the last decade. The 2 cases to be described occurred in the material from 14,000 thyroid operations.

A man, 54 years of age, was admitted because of swelling of the neck of 5 years' duration. The thyroid was bilaterally markedly enlarged. A right lobectomy was performed. The capsule was intact and the lobe enlarged to 11 cm. in diameter. Histologically, there was a diffuse, dense infiltration by plasma cells, including giant forms. Radiation therapy was given and the remaining left lobe decreased markedly. Ten years later there was recurrence on the left due to an infiltrating plasma cell tumor. The lobe was removed piecemeal owing to marked capsular and muscle infiltration. Postoperatively, 3750 r. of radiation was given. The patient died of undetermined cause 6 years later, 21 years after the apparent onset of symptoms.



The second patient was a man, 71 years of age, with swelling of the face and neck of 6 weeks' duration. There was a marked enlargement of the left thyroid lobe with substernal extension. A left lobectomy was performed. The lobe was 12 cm. in diameter, weighed 240 gm., and had a thickened, shaggy capsule. The tissue was of soft consistence and yellowish white with irregular strands of lighter stroma. In most sections there was a diffuse infiltration of the thyroid by plasma cells, including rare giant forms. The thyroid parenchyma was markedly reduced, consisting of a few small oxyphilic follicles. Fibrous trabeculae and foci of lymphocytes were present in areas. Postoperatively, radiation therapy was given, totaling 1700 r. The patient was alive and well 5 years after operation.

Both patients were men, in the first instance without evidence of precedent thyroid disease, and in the second with areas in the thyroid suggestive of struma lymphomatosa (Hashimoto). Plasmocytoma of the thyroid, like other extramedullary types, may pursue a long course and show immediate response to radiation therapy, though in one of these cases there was recurrence after 10 years. In its first appearance the neoplasm revealed no penetration of the thyroid capsule, but with recurrence there was extension to adjoining muscle. In one instance the tumor was bilateral and in the other unilateral.

#### NECROSIS OF THE LIVER IN EXPERIMENTAL FATAL HEMOGLOBINURIC NEPHROSIS.

Joseph J. Lalich (by invitation) and D. Murray Angevine, Madison, Wis.

*Abstract.* Variable degrees of hepatic necrosis have been observed in rabbits which died of hemoglobinuric nephrosis following starvation and dehydration and in addition received intravenous injections of hemoglobin. A total of 1.8 gm. per kg. of hemoglobin was given in 9 divided doses during the last 3 days of dehydration. Fifty-one rabbits have been treated in this manner. In this series, 17 of 18 rabbits died with severe hemoglobinuric nephrosis within 2 to 14 days following the first injection of hemoglobin. Of the 34 surviving animals, all had pigment casts in the renal tubules from 8 to 14 days after the initial hemoglobin injection. Hepatic necrosis was observed in 12 of 17 rabbits which died, but was not observed in animals which survived the injections.

Hemoglobinuric nephrosis was considered severe when 100 or more pigment casts were present in 10 low-power fields. The hepatic necrosis varied considerably in extent. In 4 instances the areas of necrosis were confluent and 25 per cent or more of the liver was involved. From 8 to 20 areas of necrosis per cm.<sup>2</sup> of section were seen in 4 livers and 1 to 3 areas per cm.<sup>2</sup> in 4 others. The liver necrosis may occur focally; however, in most instances it is central. Additional alterations frequently observed were congestion of the central vein and liver sinusoids, dilatation of lymphatics, and vacuolization of cytoplasm of liver cells. Increased pigment deposition was observed also, either in the liver cells or the Kupffer cells, in approximately one-half the instances in which hepatic necrosis occurred.

It is not apparent from these studies what factors are responsible for the hepatic necrosis. The observation that 5 rabbits died of hemoglobinuric nephrosis without evidence of liver necrosis suggests that the renal changes occur first. This study corroborates previous observations that dehydration and starvation are precipitating factors for the development of hemoglobinuric nephrosis, and further suggests that the same factors are probably concerned in the production of hepatic necrosis.

#### COMPLEMENT FIXATION IN ANIMAL NEOPLASIA. III. COMPARISON OF THE KIDD TECHNIC WITH A MORE SENSITIVE METHOD FOR TESTING THE SERA FROM RABBITS BEARING THE BROWN-PEARCE CARCINOMA. Stuart W. Lippincott and (by invitation) Lester D. Ellerbrook, Helen Thornton, and Ellery C. Stowell, Seattle, Wash.

*Abstract.* Kidd has reported on a complement-fixing antibody in the sera of certain rabbits of various breeds inoculated with the Brown-Pearce carcinoma. His

report has been substantiated by our studies and in addition a more sensitive and quantitative method for measuring the antibody has been devised. Definite improvements in standardizing the technic have resulted from using the amount of complement giving 50 per cent hemolysis as the unit instead of the 100 per cent unit. Optimum conditions of the test have been investigated with respect to methods of preparation and purification of the antigen, use of the proper ratio of red cells and hemolysin, and time and temperature of fixation.

Sera were obtained before inoculation of the neoplasm and at daily or weekly intervals thereafter up to 100 days. Other control sera were obtained from non-tumor-bearing rabbits from those inoculated with autoclaved and frozen Brown-Pearce tumor, with normal rabbit tissue, and with such substances as human albumin and turpentine. Some of the antigens prepared from the neoplastic tissue were the following: saline extracts, buffered saline extracts, and sediment and supernate from the saline extract centrifuged in a Sharples supercentrifuge for 60 or 90 minutes at 45,000 r.p.m. (50,000 g. at the periphery).

A number of postinoculation sera giving negative reactions by Kidd's technic gave perceptible reactions when his conditions of fixation were changed from 2 hours at room temperature to 18 hours at 5° C. Some sera which were negative by the latter procedure gave positive reactions by our standardized technic, *i.e.*, fixation for 18 hours at 5° C. in the presence of graduated known amounts of complement. The extent of the reaction was measured by the difference between the amounts of complement required to give 50 per cent hemolysis in the test and in the controls. The pre-inoculation sera showed no definite fixation of complement whereas most sera showed a definite positive reaction after the neoplasm had grown for 2 to 3 weeks.

**THE INCIDENCE OF POST-TRANSFUSION SERUM HEPATITIS.** John J. McGraw, Jr. (by invitation), Max M. Strumia, and Mary Elizabeth Burns (by invitation), Bryn Mawr, Pa.

*Abstract.* Serum hepatitis (homologous serum jaundice) is transmitted by injection of human blood and certain fractions of blood. The incidence of hepatitis following transfusions of blood and plasma is a matter of considerable importance in transfusion therapy. An attempt has been made to determine the incidence of serum hepatitis at the Bryn Mawr Hospital during a period of approximately 4 years starting in December, 1943. Complete records of all blood donations, plasma pools, and transfusion were available. The pools contained plasma of 8 to 12 donors. Letters and questionnaires were sent to 1,523 transfused patients. Identical letters and questionnaires were sent to 1,300 non-transfused patients who were admitted to the hospital in the same period. The latter were used as controls. The questionnaire simply asked if the patient developed jaundice in a period dating 6 months from the time of hospitalization.

Nine hundred and thirty-six transfused patients and 1,000 controls responded. The answers were analyzed, hospital records reviewed, and the attending physicians were contacted. Quite a number of the affirmative answers in both series were discarded because the jaundice referred to by the patient was present at the time of admission to the hospital. These were eliminated and all cases of jaundice which had their onset in the 6 month study period were classified as "possible serum hepatitis" even though some patients were known to have reason for bile duct obstruction. Further study permitted elimination of some cases as almost certainly due to common duct stones, carcinoma of the pancreas, portal cirrhosis, etc. The remaining cases were classified as "probable serum hepatitis."

In the 936 transfused patients there were 20 cases (2.12 per cent) classified as "possible hepatitis." Twelve cases (1.29 per cent) were considered as probably

due to the transfusions. In the 1,000 controls there were only 2 cases (0.2 per cent) of "probable hepatitis" in the 6 month study period.

The cases were further analyzed to determine the incidence of serum hepatitis following whole blood as opposed to pooled plasma. As might be expected, the rate was higher in those patients who received pooled plasma. In the group of 382 patients who received nothing but whole blood, there was only one case of "probable hepatitis" (0.26 per cent). In the group of 528 patients who received pooled plasma, with or without blood, there were 11 cases (2.08 per cent) of probable hepatitis.

Our findings of a 1.29 per cent incidence of probable hepatitis following blood or plasma and a 2.08 per cent incidence following plasma with or without whole blood are based upon the number of cases of hepatitis per transfused patient. Since many patients received multiple units of blood plasma, the incidence of hepatitis per transfusion is much lower. The 936 patients who responded to the survey received a total of 3,723 transfusions. The incidence per transfusion of blood or plasma was approximately 1 in 300. In the group who received Bryn Mawr plasma there were 2,350 such transfusions and the incidence of hepatitis was 1 for each 235 units administered.

Although the findings revealed in this survey indicate that serum hepatitis is a rather rare complication of plasma transfusion when the pools are relatively small, it is planned to sterilize our plasma with either ultraviolet light or nitrogen mustard and, after a period of a few years, repeat the survey on a new group of patients.

#### TISSUE CHANGES CLOSELY RESEMBLING AMYLOIDOSIS IN SCORBUTIC GUINEA-PIGS.

Conrad L. Pirani (by invitation) and Chauncey G. Bly (by invitation), Chicago, Ill.

*Abstract.* In the course of a pathologic study of chronic scurvy, deposits of amyloid-like material were observed in various organs.

Thirty young guinea-pigs were fed scorbutogenic diets for varying lengths of time; 12 received the Sherman-LaMar diet as modified by Rinehart, and 18 the Crampton no. 5 diet. The control animals received 2 mg. of ascorbic acid daily, the acute scurvy animals none, and those of the chronic scurvy group 0.2 mg. Amyloid-like material was found only in the chronic scurvy guinea-pigs and in only those animals which were sacrificed after 8 weeks or longer from the beginning of the experiments. Seven of 8 animals in this last group showed distinct deposits of amyloid-like material. In these cases the spleen was severely affected, the liver moderately, and the adrenal cortex only minimally. No other organ appeared to be involved.

Microscopically, this material appeared in the spleen in the form of a thick band in the peripheral portions of the malpighian corpuscles. In the liver it appeared in moderate amounts between the hepatic cords and the wall of the sinusoids with resulting compression and atrophy of the liver cells. In the adrenal cortex small amounts were noted in close apposition with and at times completely surrounding the walls of capillaries. Iodine and sulfuric acid tests on the gross specimens, and crystal violet and congo red stains on the sections, were all positive. The tinctorial response of this amyloid-like substance with congo red, however, was not as brilliant as is usually seen in pathologic specimens from man. In sections stained with congo red and examined with polarized light, birefringence was observed in focal areas where the amyloid-like deposits were deeply stained. The organs involved, the typical distribution of this substance in the immediate neighborhood of capillaries and of reticulo-endothelial elements, and the results of the tests with special stains are those usually observed both in human amyloidosis and experi-

mental amyloidosis in animals. No definite conclusions, however, can be reached at this time as to the identity of the substance observed in these experiments with human amyloid.

THE EXFOLIATIVE CYTOLOGIC DIAGNOSIS OF CENTRAL NERVOUS SYSTEM LESIONS.  
William R. Platt, Camden, N.J.

*Abstract.* Using the same fundamental concept described by its earliest proponents, *i.e.*, that many malignant tumors will ultimately desquamate tumor cells onto a presenting free surface, the author examined the aspirated fluids obtained from the lateral ventricles, cisterna magna, spinal canal, and cystic brain masses from patients with proved and with clinically diagnosed central nervous system entities. The microscopic patterns of these are to be presented and discussed.

EXPERIENCE WITH UNSUCCESSFUL ATTEMPTS TO USE THE ANTERIOR CHAMBERS OF EYES OF HETEROLOGOUS SPECIES TO GROW MALIGNANT NEOPLASMS AS A SOURCE OF ANTIGEN. Walter Ricker (by invitation), Seattle, Wash.

*Abstract.* The possibility of growing malignant neoplasms in the anterior chamber of guinea-pig and rabbit eyes has been investigated as a possible source of neoplastic tissue to be used for the preparation of antigens in a study of complement fixation in animal neoplasia. In an experience of 2 years the method has been found unsuitable for this purpose. Seven types of malignant neoplasms were used for transplantations. The eyes of the alien species into which transplants were made were examined for gross evidence of growth throughout the experiments, and at death or sacrifice each eye to which transplantation had been done was removed, fixed in formalin, embedded in paraffin, sectioned at 6  $\mu$ , and stained with hematoxylin and eosin. These sections were examined microscopically for residual or growing tumor. The neoplasms transplanted were: (1) Two  $C_3H$  mouse mammary carcinomas transplanted to 19 guinea-pig eyes and 21 rabbit eyes and observed for a period of 80 days. (2) Five Brown-Pearce rabbit carcinomas transplanted to 42 guinea-pig eyes, 2 of which were retransplanted to 4 other guinea-pig eyes and observed for a period of 69 days. (3) Three adenocarcinomas of human breast origin transplanted to 5 rabbit eyes and 22 guinea-pig eyes, with 1 subsequent retransplant to 1 guinea-pig eye and observed for a period of 212 days. (4) One spontaneous squamous cell carcinoma of mouse skin transplanted to 3 guinea-pig eyes and observed for a period of 192 days. (5) One spontaneous mouse hepatoma transplanted to 4 guinea-pig eyes and observed for a period of 189 days. (6) One spontaneous adenocarcinoma of cat breast transplanted to 3 guinea-pig eyes and observed for a period of 102 days. (7) Two transplanted "L" strain fibrosarcomas to 49 guinea-pig eyes and observed for a period of 103 days.

Six eyes to which the  $C_3H$  mammary carcinoma was transplanted and 5 eyes to which the Brown-Pearce carcinoma was transplanted showed proliferative changes interpreted as consistent with gross growth. Twenty-eight of the 49 guinea-pig eyes to which the "L" strain fibrosarcoma was transplanted showed changes consistent with gross growth. Of this total of 39, 4 of the eyes to which the "L" strain fibrosarcoma had been transplanted showed, on the 8th day following transplantation and by microscopic examination, neoplastic growth consistent with that observed in sample sections of the original neoplasm. All other microscopic findings were exudative or proliferative, predominantly the latter.

Of 173 transplants of 7 types of malignant neoplasms, 147 to the anterior chambers of guinea-pigs' eyes and 26 to the anterior chambers of rabbits' eyes, 39 showed changes at some interval considered consistent with gross growth of the transplant. Microscopic examination of all of the eyes corroborated the gross observation in only 4 instances in the "L" strain fibrosarcoma.

METHYLCHOLANTHRENE-INDUCED TUMORS IN GUINEA-PIGS WITH TRANSIENT PERIODS OF VITAMIN C DEFICIENCY. William O. Russell and (by invitation) Leonard R. Ortega, Houston, Texas.

*Abstract.* Because of the infrequent use of the guinea-pig in cancer research and the fact that these animals are generally believed to be refractory to carcinogenic agents, these experiments were undertaken to further evaluate this animal with regard to experimental carcinogenesis. Ninety-eight young, but mature, male and female guinea-pigs were injected subcutaneously in the right axilla with 30 mg. of methylcholanthrene dissolved in sesame oil. The animals were divided into 3 groups for the experiment. One group of 28 animals was a normal control and received a full diet, a second group of 31 animals received a full but restricted diet to control inanition, and a third group of 39 animals received periodically a scorbutogenic diet.

Seventy-three guinea-pigs lived past the appearance of the first tumor (170 days). Thirty-nine of these developed tumors at the site of the methylcholanthrene injection. Seventeen of the tumors were fibrosarcomas, 10 of them were osteogenic sarcomas, and 12 were liposarcomas. Although many of the tumors showed extreme anaplasia and evidence of rapid growth, metastases were not observed.

Fifteen tumors were produced in the scorbutic group, 12 in the inanition control group, and 12 in the normal controls. The average induction period for each group was as follows: 242 days in the scorbutic animals, 295 days in the inanition control animals, and 393 days in the normal controls. There was no observable difference in the histologic types seen in the 3 groups. The length of the induction period was remarkably shorter in the scorbutic group. These results are not statistically significant in regard to a possible effect of vitamin C deficiency on influencing the induction of the tumors. The shortened induction period in the scorbutic and inanition group is statistically significant.

OLD ENDOCARDITIS IN THE HEARTS OF PIGS RESEMBLING HUMAN ENDOCARDITIS.

Otto Saphir and (by invitation) Martin Lowenthal, Chicago, Ill.

*Abstract.* In a study of 950 hearts of pigs, old endocarditis was encountered in 10 per cent. These resembled grossly and microscopically old endocarditides in humans, often interpreted as the result of rheumatic fever. Since *Erysipelothrix rhusiopathiae*, *Salmonella suispestifer*, swine plague, and hog cholera infections with resulting endocarditis occur in a relatively high percentage of pigs, it is very likely that the old endocarditis encountered here is the result of a healed endocarditis caused by one of the above organisms, the most frequent being *Ery. rhusiopathiae*. Since this healed endocarditis is similar in many respects to human rheumatic endocarditis, the question is raised whether some instances interpreted as rheumatic endocarditis are not the result of a healed bacterial endocarditis of unknown origin.

AN EVALUATION OF THE ADAMSTONE-TAYLOR FROZEN SECTION TECHNIC FOR USE IN ROUTINE SURGICAL PATHOLOGY. Edgar B. Taft (by invitation), Kansas City, Kansas.

*Abstract.* A method for the rapid preparation of permanent, thin, frozen sections was described recently by Adamstone and Taylor (*Stain Technol.*, 1948, 23, 109-116). With this technic, sections of fresh, unfixed tissue are made and kept frozen until they are attached to microscopic slides, ready for processing. There are several differences from the ordinary frozen section technic: The microtome knife is cooled with blocks of dry ice; the sections are transferred from the knife blade to slides by means of a hollow, shovel-shaped scoop filled with

shaved dry ice; and the slides are immersed in fixative just as the tissue sections began to melt.

An old Leitz sledge-type microtome and a clinical frozen section microtome head were combined and adapted to the technic as described by its originators. Fresh tissue was obtained from the operating room or from the Pathology Department, usually within 4 hours of the time at which it was removed from the patient. Sections were made from blocks of the usual size, sometimes simultaneously from two organs such as an ovary and oviduct, and fixed in a variety of standard fixatives. Sections so obtained could be fixed, stained, and mounted permanently within 15 minutes from the time the block of tissue was frozen and doubtless could have been treated even more rapidly if it had been desirable.

There are a number of disadvantages inherent in this method. The most obvious is that sections cannot be made if the room temperature exceeds 65° to 68° F. In the winter this difficulty is easily overcome; in warm weather, even with air-conditioning or a cool basement room, such a low temperature might not be achieved readily. Considerable manual dexterity is necessary for this technic, and two persons must participate in making the sections. Bone and calcified tissue, of course, cannot be examined by this method. It also has been found that tissues with a large amount of fat, such as a lipoma or very fatty breast, do not section satisfactorily. Firm tissues with moderate to large amounts of dense collagen such as cervix uteri or leiomyoma from postmenopausal patients likewise have been sectioned with difficulty. Scirrhus carcinoma of the breast, on the other hand, has yielded excellent sections.

The advantages of this method are numerous: Permanent, thin (4 to 6  $\mu$ ), flat sections are obtained; sections for possible fat stains or histochemical determinations can be cut from the same block as the routine sections and at the same time; and sections of such tissues as lymph node, intestinal tract and spleen can be made with ease. However, it must be admitted that the most satisfactory sections are obtained from such tissues as liver, brain and kidney, so that, except in a few instances, this method is perhaps best adapted for use in experimental laboratories.

HISTOLOGIC OBSERVATIONS ON THE SUPRADIAPHRAGMATIC PORTION OF THE VAGUS NERVES. C. G. Tedeschi and E. A. Gaston (by invitation), Boston, Mass., and Framingham, Mass.

*Abstract.* The purpose of this communication is to present the microscopic findings in specimens of vagus nerve removed at operation from patients with duodenal ulcer, and in control series from post-mortem material. Reports of nerve injuries frequently include evidence of damage to peripheral nerves without loss of anatomic continuity. A spindle-shaped neuroma, a perineural thickening, a dense intraneural fibrosis or scarring is often found under such circumstances and to these lesions is traced the lasting loss of excitability. This gamut of changes and occasionally neurolysis and inflammatory cell infiltration were shown, with unexpected frequency, by the vagotomy specimens in our series. These changes were focal in distribution, the whole resulting in an irregular alternation often in the same microscopic field, of apparently intact fascicles and of fascicles displaying one, or a combination, of the above outlined changes.

A number of investigators have shown the susceptibility of the peripheral nerves to minimal trauma, including pressure and transient stretching. The nature of the changes encountered in our series speaks against the possibility that they resulted from stretching or pressure applied to the neural structures at the time of operation. Neuronal changes similar to those seen in the segments of vagus nerves removed at operation from patients with duodenal ulcer were occasionally seen in corresponding segments of vagus nerves obtained at autopsy in the absence of any

evidence of gastro-intestinal disease. The number of specimens studied up to the present time, 19 surgical and 32 post-mortem, is too small to be statistically significant. However, a comparative analysis of the findings in the surgical and in the control series revealed changes to be much more frequent in the former. Assuming that the normal function of a nerve is dependent upon the proper transmission of impulses, the possibility must be entertained that changes in the vagal nerves, such as those seen in some of our cases, may play some part in the development of disorders of structure or function occurring in the alimentary tract.

**ACID-FAST INTRANUCLEAR INCLUSION BODIES IN LEAD POISONING.** M. Wachstein, Brooklyn, N.Y.

*Abstract.* Intranuclear inclusion bodies occur after the application of various metallic compounds. Their unexpected presence in tissue sections may occasionally lead to the correct interpretation of an unexplained death. This is shown by the following observation.

A 21-month-old boy had shown increasing irritability for 4 weeks. Forty-six hours before his death he was admitted because of prolonged vomiting. During his stay in the hospital he developed drowsiness, signs of meningeal irritation, and died in coma. Significant laboratory findings consisted of an increased amount of protein (258 mg. per cent) in the spinal fluid with a cell count of 25, of which 90 per cent were polymorphonuclear leukocytes and 10 per cent lymphocytes, and evidence of considerable anemia with a few basophilic stippled red cells.

Gross autopsy findings were essentially negative. In sections of the kidneys and to a lesser degree in those of the liver, innumerable acidophilic intranuclear inclusion bodies were found. Chemical tests performed on the basis of these findings in a composite sample of kidney and liver showed 1.5 mg. of lead in 100 gm. of wet tissue (upper limit 0.05 mg. in control material).

Simular inclusion bodies, first described by Blackman, were seen in rats given a lead-containing diet. All of these inclusion bodies were acid-fast. Among various other inclusion bodies examined, only those following bismuth application proved to be likewise acid-alcohol resistant to decolorization after staining with carbolfuchsin. The renal inclusion bodies following bismuth application are, however, easily distinguished on the basis of their different morphologic characteristics.

**INFARCTION OF THE MUSCLE BUNDLES OF THE ATRIA AND VENTRICLES OF THE HEART.** William B. Wartman and (by invitation) John Souders, Chicago, Ill.

*Abstract.* Fifty hearts with infarcts were examined by cutting each like a loaf of bread from the apex to the base by means of multiple parallel transverse cuts. The distribution of the infarcts was compared with the known distribution of the cardiac muscle bundles. In every instance the pattern of the infarct coincided with the pattern of either a single muscle bundle or of a group of bundles. The entire muscle bundle was never involved but only a segment of it. Infarcts occurred in the superficial muscle bundles, either singly or in combination, in half the cases. In nearly one-fourth of all cases both superficial bundles were affected. The superficial sinospiral muscle contained infarcts twice as commonly as the superficial bulbospiral muscle. In 10 cases the superficial bundles were involved in conjunction with deep muscles. Thus the superficial muscle bundles were the seat of infarcts, either singly or in combination, in 71 per cent of cases. Deep muscle bundles were affected 22 times (48 per cent). In 6 cases both deep muscles were affected and in 10 cases deep muscles were affected in combination with the superficial muscles.

Infarcts were found in the atria in 14 hearts (29 per cent). Most of these were in the right auricle and were recent, suggesting that they may have contributed to the death of the patients.

It was found in general that when the right coronary artery was occluded an infarct developed in a deep muscle; when the descending branch of the left coro-

nary artery was occluded an infarct developed in one or both of the superficial muscles. When the circumflex branch of the left coronary artery was occluded the infarct developed in either deep or superficial muscles. When multiple occlusions occurred in different coronary arteries there was great likelihood that more than one muscle bundle would be the seat of infarction.

It was possible to classify infarcts into three types depending upon the thickness of the ventricular wall which was involved. Type 1, or full thickness infarcts, included those in which the entire thickness of the ventricular wall was necrotic. Type 2, or massive infarcts, involved a large mass of muscle, but not the full thickness of the ventricular wall. Type 3, or laminar infarcts, involved only a portion of a single muscle. Full thickness and massive infarcts occurred with nearly equal frequency (approximately 36 per cent for each type). The remainder (approximately 25 per cent) were laminar. Rupture occurred only in hearts with full thickness infarcts. However, the fact that not all hearts with full thickness infarcts ruptured indicates that other factors, such as blood pressure, and the degree of liquefaction of the infarct, were also important. Ninety per cent of cardiac aneurysms occurred either with full thickness or massive infarcts and only 10 per cent with laminar infarcts.

When the infarct was of full thickness type, two or more bundles were involved in most cases (13 of 17). Massive infarcts involved two or more bundles in 13 instances and a single muscle in 7. The situation was reversed in the case of laminar infarcts. There were 8 in which a single muscle was affected and only 3 in which 2 muscle bundles were involved. Fifty per cent of the hearts had an old infarct and of these all but 4 also had a new infarct. A striking feature was the frequency with which the new infarct occurred in the same area as the old one.

**BILATERAL MAMMARY ARTERITIS.** Theodore R. Waugh, Montreal, Canada.

*Abstract.* A case is reported of a 63-year-old woman who developed a lump in the right breast due to an inflammatory condition of a branch of the mammary artery. This was followed 2 months later by a similar lesion of the left breast. The histopathologic changes in the vessels and clinical course of the disease are similar to those met with in temporal arteritis.

**COLOR REACTIONS WITH SERA OF PATIENTS WITH MALIGNANT NEOPLASMS.** Emil Weiss, Chicago, Ill.

*Abstract.* A number of aniline dyes in dilutions of 1:10,000 do not show any color changes on addition of sera from patients with cancer. Sera of normal individuals and of those afflicted with various diseases or benign tumors change to green the original blue color of the same dyes. The glassware must be clean and free of any acid or dye. The sera must be fresh and thoroughly centrifuged. Turbid, hemolyzed, or icteric sera cannot be used. The dyes are kept in 0.1 per cent stock solutions of rubbing alcohol (70 per cent isopropyl alcohol). The stock solution is diluted with distilled water 1:10 before use. To 0.3 cc. of the diluted dye 0.5 cc. of serum is added, the tube is shaken for a few seconds, and the color changes are noted. A positive control containing a known malignant serum and a negative control containing a known normal serum are handled in the same manner. A dye control, correspondingly diluted with water, also is used. The tubes are placed overnight or for 12 hours in the refrigerator and then the final reading is made. Eventual color changes become more distinct after standing several hours. Over 300 sera were examined with the above technic. Positive (blue) reactions were obtained in 89.1 per cent of sera from patients with malignant neoplasms. Azur II, toluidine blue, trypan blue, and Victoria blue were found to be equally useful. This method applies to all types of cancer. Hodgkin's disease and various forms of leukemia give consistently positive results.





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## THE RESPONSE OF TISSUE TO TOTAL BODY IRRADIATION\*

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It has been fairly well established that, provided ionization within tissue is achieved, the biologic effects brought about by any of the several types of ionizing radiations are essentially similar.<sup>1-4</sup> Thus the effect produced by penetrating external radiations is similar to the effect of internally deposited materials, provided equal amounts of roentgen units or roentgen equivalents are delivered to the tissue.

Any cell will be destroyed if it receives a sufficiently large dose of ionizing radiations. However, for reasons unknown, there are marked differences in the sensitivity of the various cells of a given species to irradiation; and, even among cells of a single type in different stages of development there are marked differences in radiosensitivity. In general in a given cell series, the cells in the early stages of mitotic division and blast cells are radiosensitive while mature cells are more radioresistant.

It is of special interest that reticular cells of lymph nodes and bone marrow are relatively radioresistant. This fact has not been emphasized in the literature, but seems to be quite clear from histopathologic observations. For instance, in swine that have received heavy doses of total body irradiation, reticular cells in the lymph nodules are prominent among the surviving cells. The radioresistance of reticular cells plays an important part in the recovery of borderline cases that receive heavy doses.

It has been reported that the radioresistance of various mature cells increases in approximately the following order: lymphocytes, germ cells, granulocytes, epithelium, endothelium, muscle, connective tissue,

\* The opinions or conclusions contained in this report are those of the author. They are not to be construed as necessarily reflecting the views or the endorsement of the Navy Department.

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bone, and nerve cells.<sup>5,6</sup> In these cells all degrees of injury may be observed, depending on the stage of development of the cells at the time of injury, the degree of ionization accomplished, and probably other factors.

The problem of interpretation of biologic response to radiation is further complicated by differences in species sensitivity. Guinea-pigs, for instance, are radiosensitive while rats are relatively radioresistant. Goats and swine fall somewhere between the two, within what is estimated as the sensitivity range of man.

Finally, it is important to grasp the concept of total body irradiation in contrast to localized irradiation. An amount of radiation that is well tolerated when given locally might be sufficient to kill if administered simultaneously to the total body. For instance, it is estimated that 300 to 600 r. of total body irradiation is the range of lethal dosage for man, yet doses up to 10,000 r. may be given to a small tumor with no general reaction whatsoever; and doses up to 400 r. may be given to an area up to 20 by 20 cm. with no more than transient radiation sickness characterized by nausea and perhaps vomiting.

#### MATERIALS AND METHODS

One group of swine discussed in this paper was exposed to total body irradiation from atomic bomb explosions at Bikini in the summer of 1946. The Naval Medical Research Section of Joint Task Force One placed the animals on target ships at various distances from the bomb explosion. The test positions afforded degrees of shielding from ionizing radiations varying from very little to the amount of protection supplied by 6 or 8 inches of steel. The animals were recovered as soon after the tests as practicable and a few survivors are still under observation. The biologic experimental design is more fully described in other reports.<sup>7,8</sup>

The second group of swine discussed in the present report was studied separately at the Naval Medical Research Institute.<sup>9,10</sup> These swine were subjected to radiations from a 1000 kv., 3 ma. x-ray source with no added filter at 1 m. distance. The experimental design permitted the delivery of measurable amounts of penetrating x-rays to the total body of 4 swine at a time at the rate of 30 r. per minute. The 50 per cent depth dose, measured in a medium of rice and sodium bicarbonate, was delivered to the approximate mid-vertical plane of the swine. In an effort to achieve uniform dosage throughout the animals, they were irradiated with the same dose on each lateral aspect in succession. The LD<sub>50</sub>/30 days was established as 275 r. measured in air, administered bilaterally<sup>9</sup> and the tissue responses to the different doses were studied.<sup>10</sup>

## RESULTS

*Mortality*

The period between exposure to ionizing irradiation and death was found to be roughly proportional to the size of the dose. Some of the animals exposed at Bikini died soon after the explosion, but owing to the experimental conditions most of them were not recovered for 1 to 5 days after the test. The animals which were dead on the target ships at the time of recovery generally had undergone too extensive autolysis to be studied profitably. However, the animals which were recovered alive could be studied carefully. Deaths occurred in these animals after a latent period of 1 to 29 days. The single death that occurred after the 30th day was that of an animal in which leukopenia had persisted from the time of irradiation and was the result of pneumonic involvement. The other late deaths probably were not caused by irradiation damage.

All but one of the swine irradiated with million volt x-rays died within 30 days or recovered.<sup>9</sup> The one survivor, which subsequently died on the 91st day after irradiation, had a consistently low white blood cell count and developed pneumonia. This swine and the Bikini swine cited are the only deaths in the two experiments so far which might be classed as delayed radiation deaths.

*Gross Pathologic Findings*

The lesions in swine exposed to lethal amounts of ionizing radiations were of three main types: hemorrhage, necrosis, and secondary infections. The similarity between the gross lesions produced by ionizing radiation from the release of atomic energy (Figs. 1, 3, 5, 7, 9, and 11) and those produced by exposure to million volt x-rays (Figs. 2, 4, 6, 8, 10, and 12) was manifest. Hemorrhages occurred as petechiae, ecchymoses, and sometimes as small hematomas. All parts of the body and all types of tissues were involved. Extravasation of blood into tissue spaces was widespread throughout the body and accounted for considerable hematic loss, but a much more voluminous loss occurred via the gastro-intestinal and urinary tracts. Surface erosions and ulcerations in the gastro-intestinal tract (Figs. 7 to 10) were gross bleeding areas which fostered external blood loss. The source of the considerable bleeding into the kidney pelves (Figs. 11 and 12) was not obvious in the sense that no ulcerations or bleeding points could be seen grossly. This hemorrhage appeared clinically as hematuria and was a sign of severe radiation illness.

The macroscopic manifestations of necrosis appeared as ulceration in

mouth, tonsils (Figs. 3 and 4), stomach (Figs. 7 and 8), and the small and large intestines. In swine, ulcers occurred more frequently in the large than in the small intestine. In all parts of the gastro-intestinal tract ulceration occurred more frequently in the Bikini animals than in those that died from million volt x-radiation, but in the mouth and tonsils, ulcers were equally prevalent in the two groups. A striking feature of the ulceration in both groups was the absence of purulent reaction. The ulcers were shallow, clearly demarcated, hemorrhagic, and free of pus.

Swine that survived the hemorrhagic and ulcerative lesions produced by irradiation frequently succumbed to secondary infections to which they were more than normally susceptible because of leukopenia, lowered resistance, anemia, and bone marrow damage. Pneumonia, usually of bilateral lobular distribution in hemorrhagic edematous lungs, was often a contributory cause of death in irradiated animals. In borderline cases which might otherwise have survived, the development of pneumonia was fatal. Other infections were uncommon. One animal had pericarditis with pneumonia and one died from a generalized gas bacillus infection. There were no generalized skin abscesses.

### *Histopathologic Findings*

The histopathologic features were similar in the two experimental groups. Those of the x-irradiated animals will be published elsewhere.<sup>10</sup> The present report deals solely with the histopathologic findings in the Bikini swine. The microscopic tissue changes which will be discussed were observed in swine which died within 14 days after exposure to total body irradiation well over 1000 r. This dose is well in excess of the absolute lethal dose.

It is assumed that the mechanism of action of ionizing radiation is to produce ionization within the cytoplasm and/or nucleus of the cell, thus disturbing its physiologic functions or chemical balance. The injury may extend to irreparable damage or only to partial damage from which total or partial recovery is possible. The greatest morphologic changes are found in the more radiosensitive cells while the radioresistant cells show little, if any, alteration following exposure to ionizing radiation.

The bone marrow and lymphoid elements underwent changes with small doses of total body radiation. When, as reported here, lethal amounts of radiation were used and the terminal picture was described, very extensive damage to the tissue was seen (Figs. 13 and 14). In addition to extravasation of blood, which occurred at random throughout the body, the outstanding feature of irradiated marrow and lymph nodes was the reduced cellularity. In bone marrow, only scattered islands of

hematopoietic tissue remained and cell reproduction in these islands often appeared stagnant. It was not uncommon to find intact only fat cells, a few clumps of erythrocytes, lining cells of blood vessels, and dispersed individual reticular cells and myelocytes (Fig. 15). Blast cells were very difficult to find in the bone marrow of animals that died of ionizing radiation injuries. The stroma assumed an opaque ground-glass appearance.

In lymph nodes, the lymphoid cells, particularly the small lymphocytes, were depleted. This was particularly noticeable in the lymph nodules in which the reticular cells assumed relative prominence in the absence of the normal lymphocyte population (Fig. 16). The large lymphocytes did not appear to be as markedly depleted as the small lymphocytes. It is interesting that vacuolation of cytoplasm or nucleus in recognizable lymphocytes or monocytes was not found. Evidently, the irradiation effect on these cells, when great enough to cause any morphologic alteration, caused pyknosis, karyorrhexis, and rapid destruction. In some of the nodes free (circulating) macrophages were increased, probably through the process of mobilization of existing fixed (tissue) macrophages. Erythrophagocytosis (Fig. 17) and an abundance of blood pigment granules, both phagocytized and not phagocytized (Fig. 18), were commonly observed. Dilatation of the lymph sinuses and edema of the pulp usually were present. The margins of the dilated sinuses tended to undergo coagulation necrosis (Figs. 19 and 20). There was no cellular response, either granulocytic or lymphoid, to the necrosis, but the lymph tended to coagulate on the necrotic lining cells of the sinus. The lacy beaded networks of fibrin, which were formed, partially occluded the sinus. The collagen in the fibers of the stromal network underwent a hyaline change and became thickened and prominent.

Lymphoid tissue in other parts of the body, particularly in the spleen (Fig. 21), the tonsil (Fig. 22), the thymus, and the intestine, showed changes similar to those described in the lymph nodes. When the dose of ionizing radiations was great enough to cause death within a few days, necrosis and reduction in numbers of the lymphocytes were seen. On the other hand, when the animal survived 10 days or longer, the fragmented and pyknotic lymphocytes had largely disappeared leaving fairly normal-appearing large and small lymphocytes in reduced numbers.

Marked congestion of the dilated blood sinuses in the spleen was an almost constant feature. This was associated after a few days with increased deposits of blood pigment and erythrophagocytosis. Lymphopoiesis was never increased and usually appeared depressed. There was no evidence of hematopoiesis in other cell lines.

In addition to the reduction in the diffuse and aggregated lymphoid elements in the tonsils, epithelial ulceration and follicular necrosis were common (Fig. 23). The necrosis did not elicit a granulocytic or lymphocytic response and seemed to occur in random follicles with no apparent reason for localization in the affected structures.

The same random location of lesions was found for mucosal ulceration in the stomach and intestine. Mucosal ulcers were more common in the large intestine than in the small intestine, and were more common in either of these locations than in the stomach or esophagus. The intestinal ulcers often were covered with a necrotic membrane (Fig. 24) and were sharply demarcated from the adjacent non-ulcerated mucosa (Fig. 25). Some of the vessels beneath the ulcers were thrombosed (Fig. 26), but this was not a constant finding. What part, if any, thrombosis played in producing ulceration can only be surmised. Vessels that did not contain thrombi were dilated and congested. Thrombosis was fairly constant and the resulting anoxia in the tissue supplied by these vessels probably materially influenced the extent of the necrosis. The submucosa usually was edematous and the cellular components were reduced in number. Large and small lymphocytes appeared to be slightly reduced in numbers in the edematous lamina propria and submucosa, but the lymphoid aggregations corresponding to Peyer's patches were markedly depleted of cells. Plasma cells were numerous and of normal appearance. The connective tissue cells of the submucosa sometimes had degenerated in a bizarre manner (Fig. 27). The affected cells became swollen and vacuolated. The collagen became an edematous, fibrillar, lacy network which spread aimlessly in the immediate vicinity.

Occasional hemorrhages occurred in the muscle and subserosa of the stomach and intestine, but otherwise these layers appeared to be uninvolved. Ulcers in the stomach were fewer, but larger and more hemorrhagic, than those in the intestine.

Evidence of damage less severe than ulceration was found in the glandular epithelium of both stomach and intestines. It took the form of distortion, variation in size, and vacuolization of the cells (Fig. 28). The differences in cell structure were apparent within the same gland as well as from gland to gland.

In the lungs, congestion of the vessels in the alveolar septa and extravasation of edema fluid into the alveoli were observed in most of the animals. The edema fluid sometimes was clear and homogeneous, but more often it contained fibrin, macrophages, and/or erythrocytes (Figs. 29 and 30). In some swine the lesions were more advanced and consisted of focal areas of necrosis and hemorrhage. A few macrophages

sometimes were seen in these areas but otherwise there was no white blood cell response to the lesions. The two features which characterized ionizing radiation injury of the lungs were (a) the great variability of the lesions in the same lung, ranging from congestion and edema to necrosis, and (b) the absence of cellular infiltration.

Some of the bronchioles contained a few desquamated epithelial cells and edema fluid, but others in the same lung appeared normal.

Focal areas of hemorrhage and necrosis were seen in the kidneys but with less than expected frequency (Fig. 31). The glomeruli appeared to be unharmed. The loops of Henle and collecting tubules were rarely involved, but the proximal and distal convoluted tubules seemed to be, to an equal degree, slightly more vulnerable to radiation injury. However, both the glomerular and tubular epithelia were highly radioresistant. Hemorrhages occurred along the course of the interlobular vessels but tended to remain localized.

The epithelium of the calyces, the pelvis, the ureters, and the bladder was more radiosensitive (Fig. 32) than that of the tubules. Congestion and hemorrhage in the epithelial and subepithelial layers were frequently observed and often were associated with vacuolization, desquamation, and necrosis of the epithelial cells. Hematuria, a common clinical observation in the heavily irradiated swine, probably was the result of lesions in the epithelium and lamina propria of the renal pelvis, ureter, and bladder. Lesions in the kidney parenchyma did not appear to contribute to the hematuria in any of the swine studied.

The male swine irradiated at Bikini were sexually immature. The normal seminiferous tubule in swine 3 to 4 months old contains only one or two rows of epithelium which consists of numerous primitive stem cells, the so-called indifferent cells, and a few spermatogonia and rare Sertoli cells. The epithelium is in the resting stage and spermatocytogenesis is not taking place. The testes of the Bikini swine that received a lethal dose of total body irradiation lost most of their spermatogonia; in fact, it was difficult to find these cells at all in many cases (Fig. 33). The indifferent cells of the seminiferous tubules exhibited no structural alterations after irradiation. Amorphous cellular debris filled the lumina of the tubules. The Sertoli cells and the interstitial tissue, except for scattered small hemorrhages, appeared unaltered by irradiation.

The sows at 3 to 4 months of age were more mature than the boars of the same age. This comment is based on the fact that an occasional corpus luteum was seen in the ovaries of 4-months-old sows while in the boars of comparable age there was no evidence of spermatogenesis. After lethal doses of total body irradiation the number of atretic follicles



was increased (Fig. 34). The chromatin in the nuclei of some of the ova was clumped. Other ova were undergoing karyolysis or had disappeared entirely. However, many ova showed no morphologic alteration and presumably remained fertile. The stroma of the ovary became edematous but the cells were unaltered. Hemorrhages in the ovary were rare.

Vacuolation of nuclei and cytoplasm occurred in the epidermis, particularly in the basal layers (Fig. 35). In some nuclei the chromatin became clumped and assumed bizarre configurations. The dermis became edematous and the collagen underwent hyaline changes. The capillaries of the dermis often were congested or plugged with agglutinative thrombi. In the hair follicles the normally clearly defined layers of epithelium lost their definition and tended to merge with one another (Fig. 36). The sebaceous and sweat glands of the skin usually appeared unchanged.

The liver, pancreas, thyroid gland, adrenals, salivary glands, epididymis, fallopian tubes, uterus, smooth muscle, striated muscle, cardiac muscle, bone, cartilage, and nerve fibers rarely showed any evidence of injury. Occasionally, hemorrhages occurred in the liver, adrenal gland, and muscle, but in the other radioresistant tissues even hemorrhage was lacking. The minimal changes that were observed in cells of the liver and adrenal glands were indistinguishable from those seen routinely in these cells in most post-mortem studies. However, it should be emphasized that no special stains were used and no microchemical analyses were made to bring out elusive alterations in the parenchymal composition of the liver and adrenal glands.

### DISCUSSION

The histopathologic changes observed after total body irradiation produce nonspecific effects similar to those brought about by any of a number of toxic agents capable of causing widespread tissue destruction and hemorrhage. There are no pathognomonic microscopic lesions in animals subjected to total-body irradiation. However, if microscopic examination reveals some degree of necrosis of the cells of the bone marrow, spleen, lymph nodes, gonads, and gastro-intestinal tract; swelling and hyalinization of collagen in connective tissue; hemorrhage and edema in tissue spaces; or erythrophagocytosis and excess blood pigment production and phagocytosis; and particularly, if a combination of these lesions is observed in association with a history of exposure to total body irradiation, a diagnosis of acute radiation injury may be made with fair certainty. Other aids in making the diagnosis include determination of the white blood count for evidence of reduction of lymphoid cells and

granulocytes, and examination of the plasma for a circulating anti-coagulant. Again, neither of these procedures gives pathognomonic findings, but both are diagnostic aids when occurring in company with other evidence of radiation injury.

A titratable anticoagulant has been reported in the blood of irradiated animals.<sup>11-13</sup> This, plus the fact that the blood of irradiated animals is incoagulable in a test tube,<sup>12</sup> poses an interesting paradox when the presence of clotted blood and fibrin in the tissues of these same animals is noted. The circulating heparin-like substance, the prolonged coagulation time of whole blood *in vitro*, and the ability to form fibrin *in vivo* do exist side by side, but the explanation is not understood.

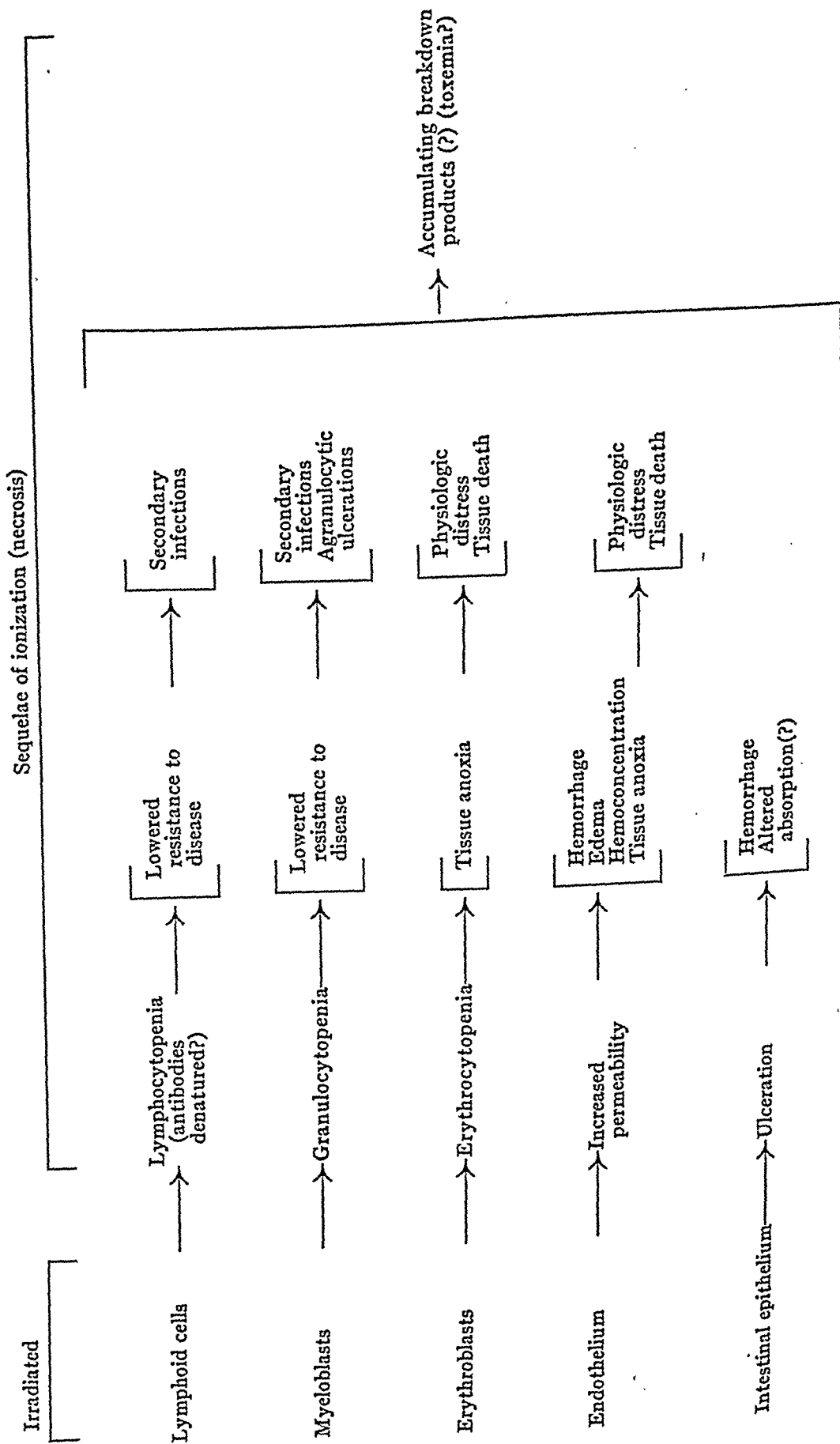
The sequence of events following total body irradiation is apparent from Table I. By destruction or injury of the hematopoietic cells of the lymph nodes, spleen, and bone marrow, the subject's resistance to infection is lowered. The cellular defense is markedly reduced and, if destruction of the lymphoid cells denatures the antibodies they carry, the humoral defense is probably reduced also.

The radiosensitive intestinal epithelium is sloughed off, leaving an ulcerated bleeding surface. If the barrier that normally prevents absorption of toxic intestinal contents is injured, then it would seem probable that toxic products would be absorbed. Against this theory, however, is the normal appearance of the liver cells, which might be expected to show some evidence of toxic necrosis if toxins were being absorbed via the intestinal tract.

The widespread hemorrhage into the tissue spaces and the edema are probably the result primarily of increased capillary permeability and secondarily of changes in the circulating blood. Dilatation of capillaries is an early and generalized reaction to radiation. Dilatation, congestion, hemostasis, increased capillary permeability, and tissue anoxia follow in logical order, much as in the pattern of reaction to shock.<sup>14</sup> Once tissue anoxia is introduced, the "vicious cycle" becomes more and more difficult to break.

It may be that the life of the heavily irradiated subject depends on the prevention of the physiologic sequences leading to tissue anoxia. Certainly, anemia and impaired circulation with resulting stagnant anoxia must play an important part in recovery or death. Anoxia and lowered resistance to infection comprise a very formidable barrier for the injured host to overcome. To these two basic sequelae of radiation injury must be added a possible third. The very nature of the injury which produces widespread necrosis suggests that there must be an accumulation of products of the breaking down of tissues. Perhaps this

TABLE I  
The Biologic Effects of Acute Radiation Injury with Reference to Selected Cells



produces a toxemia which either alone or in combination with the other two factors is responsible for radiation sickness and death. However, toxemia has not been proved chemically and the fairly normal appearance of liver and kidney parenchyma do not support the supposition.

Many assumptions must be clarified before radiation disease will be understood. There is one hopeful note that comes from these studies and that is that the reticular cells of lymph nodes and bone marrow are relatively radioresistant. As long as these building blocks remain intact it does not seem insurmountable to restore impaired circulation and bolster resistance to infection until such time as the host is again able to take over these functions without outside aid. The very severely damaged marrow will not regenerate, but in subjects with sublethal injury, provided they do not die from secondary causes, the possibility of regeneration of the bone marrow and recovery exists.

### SUMMARY AND CONCLUSIONS

The lesions produced in swine by exposure of the total body to ionizing radiations from an atomic bomb explosion are indistinguishable from lesions produced by exposure of the total body to million volt x-irradiation. These lesions are characterized by hemorrhage, necrosis, and secondary infections.

Lymphoid cells, myeloblasts, erythroblasts, germ cells, and intestinal epithelium are found to be particularly radiosensitive. Injury to these cells causes anemia and lowering of resistance to infection.

Irradiation causes dilatation of capillaries, impairment of circulation, and tissue anoxia. Anemia enhances both lowered resistance to infection and anoxia, and thus the pathologic state becomes self-perpetuating.

Absorption of toxic substances through injured intestinal mucosa and the accumulation of products of tissue destruction in the blood stream are phenomena which logically might follow the widespread necrosis that occurs after total body irradiation. There is, however, no chemical proof or histologic evidence of toxemia from either of these sources.

Since the most primitive hematopoietic stem cells—the reticular cells—are relatively radioresistant, the effort to reduce mortality does not seem altogether hopeless. Management of total body radiation disease should be directed at prevention of secondary infections and treatment of anemia, impaired circulation, and anoxia.

I am greatly indebted to Lt. Col. C. F. Tessmer, MC, USA, for valuable aid and assistance; and to J. S. Otto, HMC, USN; C. R. Helwig, HMC, USN; H. H. Marrer, HMC, USN, and D. J. Skelley, HN, USN, for valuable technical assistance in this study.

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## DESCRIPTION OF PLATES

## PLATE 115

Figures 1, 3, and 5 are photographs of lesions in swine exposed to ionizing radiations from the atomic bomb, while Figures 2, 4, and 6 are counterparts in animals exposed to million volt x-irradiation.

FIG. 1. Extensive purpura of the skin. Bikini pig 330, 13 days after irradiation.

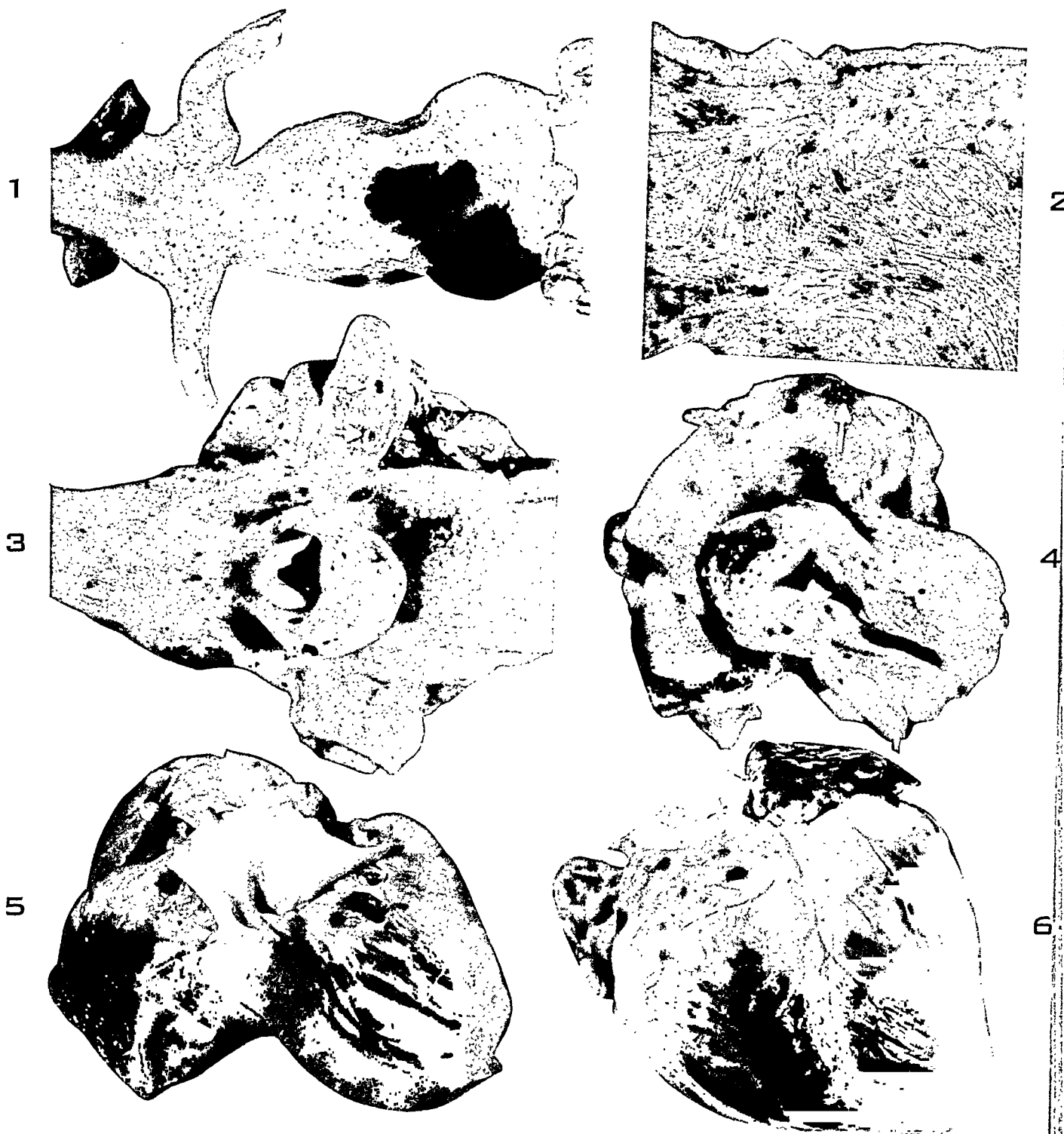
FIG. 2. Similar to Figure 1. Local pig F, 16 days after 600 r. delivered bilaterally, measured in air.

FIG. 3. Hemorrhage and edema in the oral pharynx with ulcers (arrows) in the tonsillar tissue. Bikini pig 402, 13 days after irradiation.

FIG. 4. Similar to Figure 3. Local pig 470, 13 days after 400 r. delivered bilaterally, measured in air.

FIG. 5. Hemorrhage in the myocardium of auricles and ventricles. Bikini pig 288, 11 days after irradiation.

FIG. 6. Similar to Figure 5. Local pig E, 16 days after 600 r. delivered bilaterally, measured in air.



Tullis

Total Body Irradiation

## PLATE 116

Figures 7, 9, and 11 are photographs of lesions in swine exposed to ionizing radiations from the atomic bomb, while Figures 8, 10, and 12 are counterparts in animals exposed to million volt x-irradiation.

FIG. 7. Multiple ulcers in the stomach and numerous petechial hemorrhages in the mucosa of the stomach and duodenum. Bikini pig 240, 8 days after irradiation.

FIG. 8. Similar to Figure 7. Local pig 418, 15 days after 400 r. delivered bilaterally, measured in air.

FIG. 9. Multiple hemorrhagic areas in the bowel, visible through the serosa. The mesenteric lymph nodes are enlarged, edematous, and hemorrhagic. Bikini pig 393, 11 days after irradiation.

FIG. 10. Similar to Figure 9. Local pig A, 14 days after 400 r. delivered bilaterally, measured in air.

FIG. 11. Massive hemorrhage in the pelvis of the kidneys (Bikini pig 240) 8 days after irradiation, and hemorrhages in the testicle (Bikini pig 456) 13 days after irradiation.

FIG. 12. Similar to Figure 11. Kidney from local pig XU and testicle from local pig U, 28 days after 600 r. delivered bilaterally, measured in air.

7



8



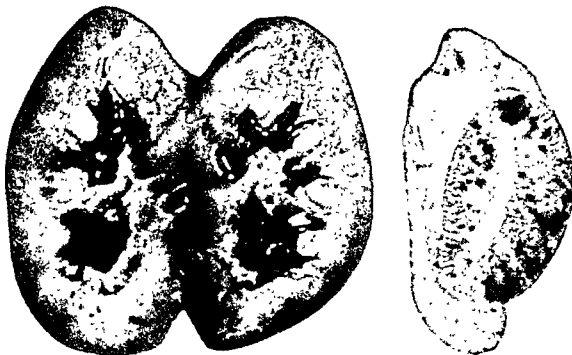
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Tullis

Total Body Irradiation



## PLATE 117

All photomicrographs—Figures 13 through 18—are taken from lesions in swine exposed to ionizing radiations from the atomic bomb tests at Bikini.

FIG. 13. Bone marrow of pig 240, 8 days after irradiation. Of note is the general washed-out appearance of the marrow and absence of hematopoiesis.  $\times 200$ .

FIG. 14. Lymph node of pig 459, 5 days after irradiation. There is diffuse hemorrhage and marked reduction in lymphoid elements.  $\times 100$ .

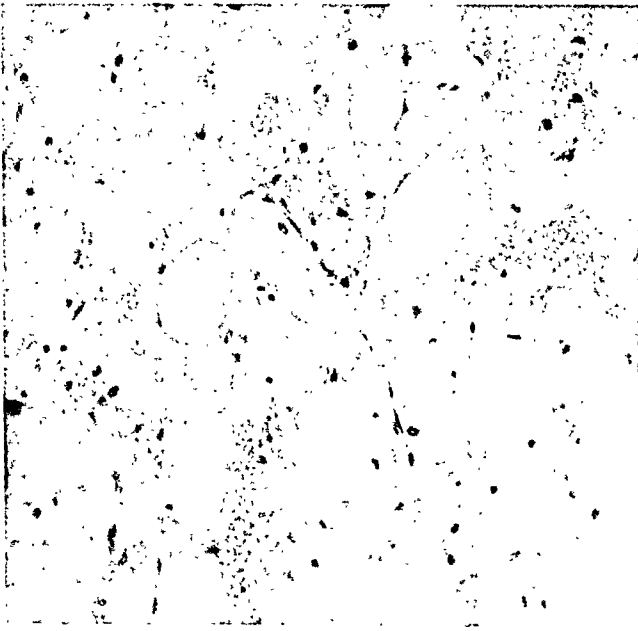
FIG. 15. Bone marrow of pig 240. In a magnified portion of Figure 13 there are five surviving, normal-appearing reticular cells and one unidentified white blood cell (myelocyte?) in addition to scattered erythrocytes.  $\times 600$ .

FIG. 16. Lymph node of pig 265, 6 days after irradiation. The lymphoid elements are depleted, causing relative prominence of reticular cells which are unharmed by irradiation. Thickening and hyaline change in the reticulum network of the nodule may be noted. A small vessel near the nodule is occluded with an agglutinative thrombus.  $\times 200$ .

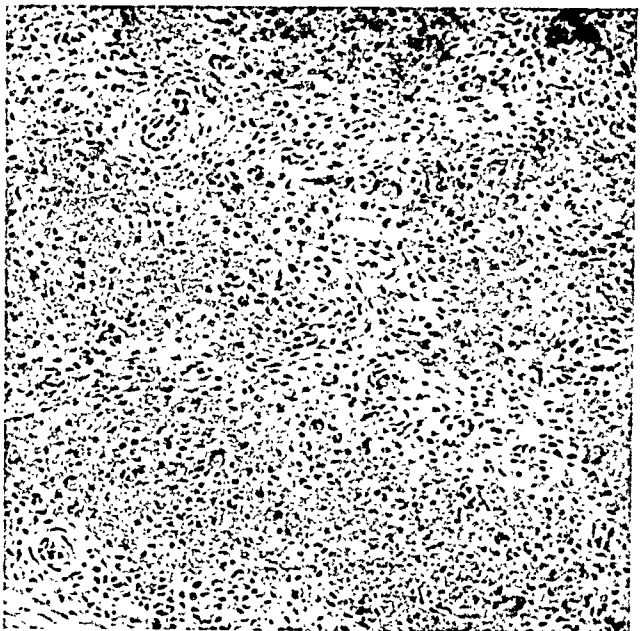
FIG. 17. Erythrophagocytosis, pig 374. Seven days after irradiation a free macrophage is in the sinus of a lymph node. There are five or six ingested red blood cells and two dark, irregular masses in the cell. The latter are ingested blood pigment granules. The nucleus is not shown.  $\times 900$ .

FIG. 18. Lymph node of pig 240, 8 days after irradiation. There has been considerable hemorrhage and destruction of blood, with scattered deposition of blood pigment granules both within and outside phagocytes.  $\times 200$ .

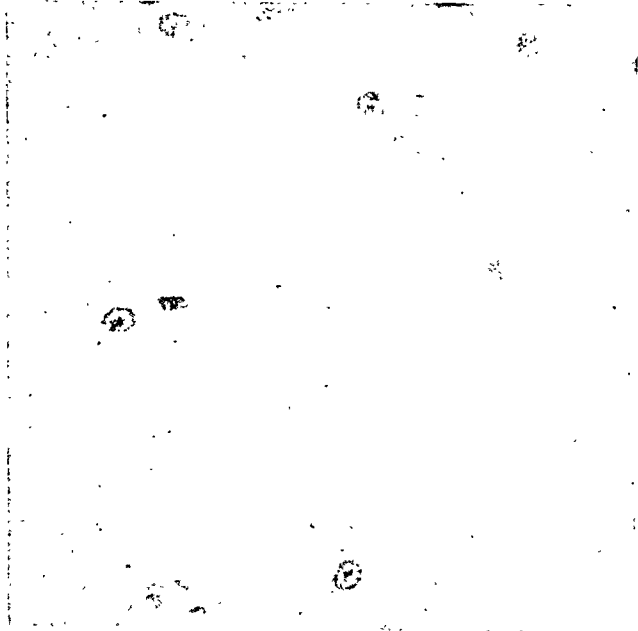
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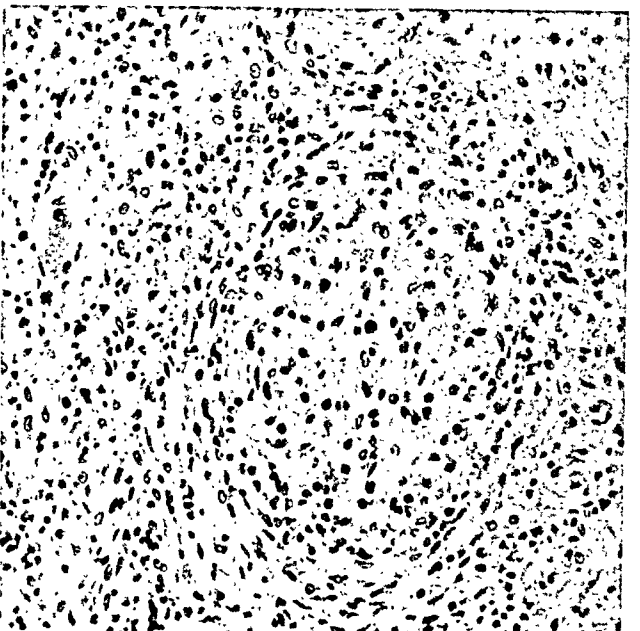
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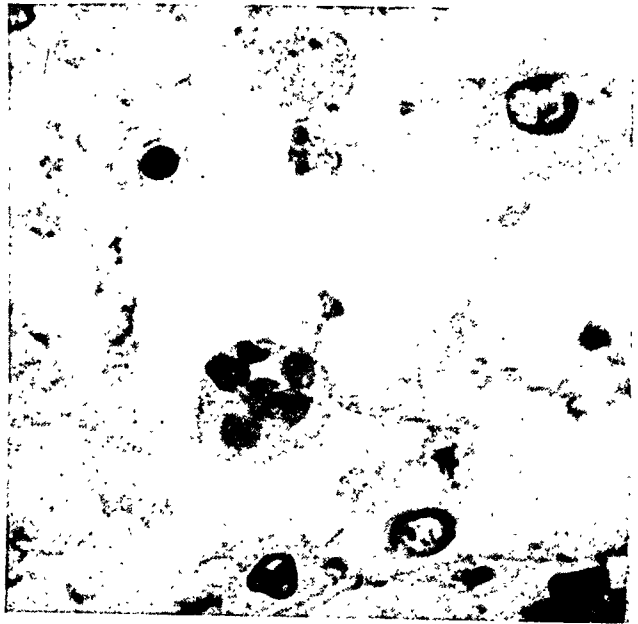
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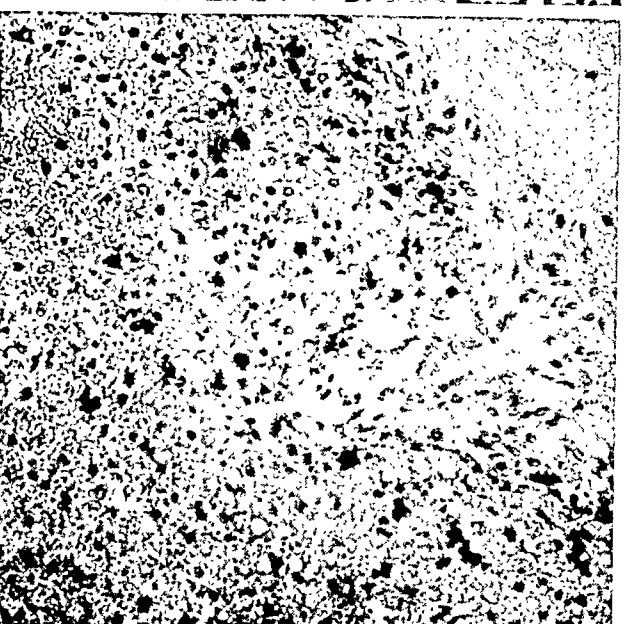
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Tullis

Total Body Irradiation

PLATE 118

All photomicrographs—Figures 19 through 24—are taken from lesions in swine exposed to ionizing radiations from the atomic bomb tests at Bikini.

FIG. 19. Lymph node of pig 265, 6 days after irradiation. The wall of the dilated sinus is undergoing necrosis of a coagulative type with a deposition of fibrin on the surface. The vessel on the right margin of the field is occluded.  $\times 200$ .

FIG. 20. Lymph node of pig 265. Higher magnification of a portion of the node in Figure 19. Detail of the fibrin deposit on the necrotic tissue is brought out. In addition, it should be noted that many of the surviving cells in the node are of the reticular type.  $\times 400$ .

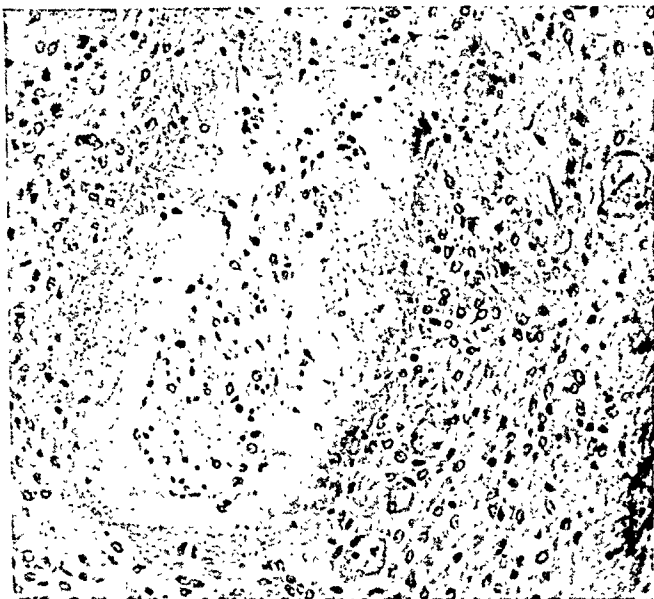
FIG. 21. Spleen of pig 459, 5 days after irradiation. There is a marked reduction in the lymphoid elements in the region of the malpighian corpuscles.  $\times 200$ .

FIG. 22. Tonsil of pig 459, 5 days after irradiation. The vessels are dilated and congested. Several of them appear completely occluded with agglutinated erythrocytes. The lymphoid elements in the stroma of the tonsillar tissue are markedly depleted.  $\times 100$ .

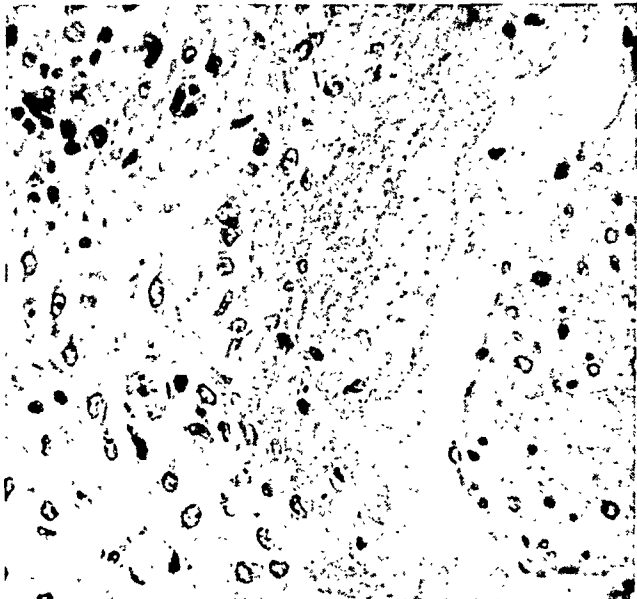
FIG. 23. Necrosis in a follicle of a tonsil of pig 240. Of note is the lack of cellular response to the necrosis.  $\times 200$ .

FIG. 24. Necrosis and ulceration in the mucosa of the large intestine of pig 459. There is not only a lack of cellular response to necrosis but the lymphoid elements normally seen in the lamina propria are depleted. Also the fibrin network covering the ulceration is nearly devoid of cells.  $\times 100$ .

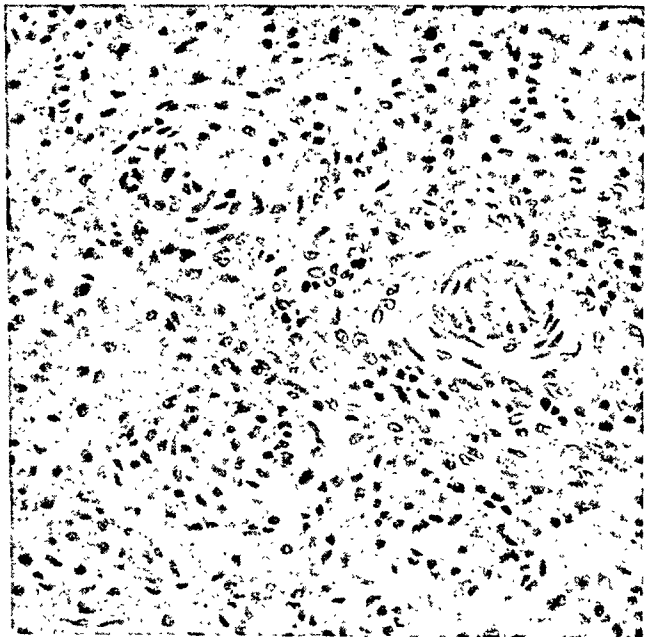
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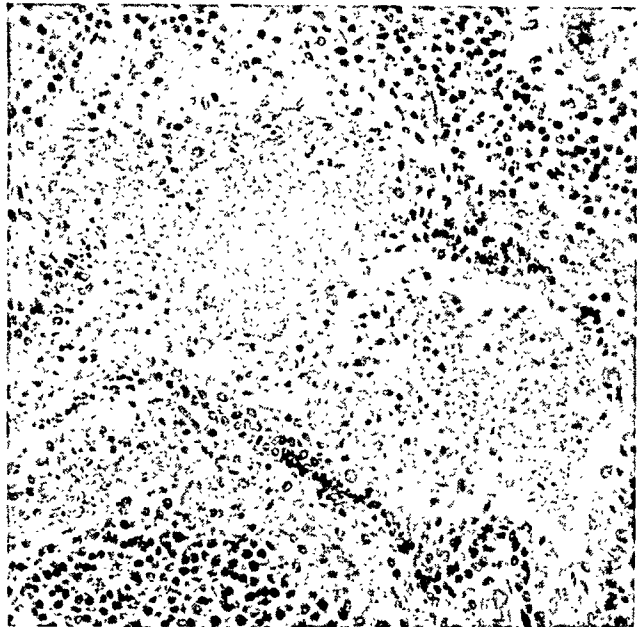
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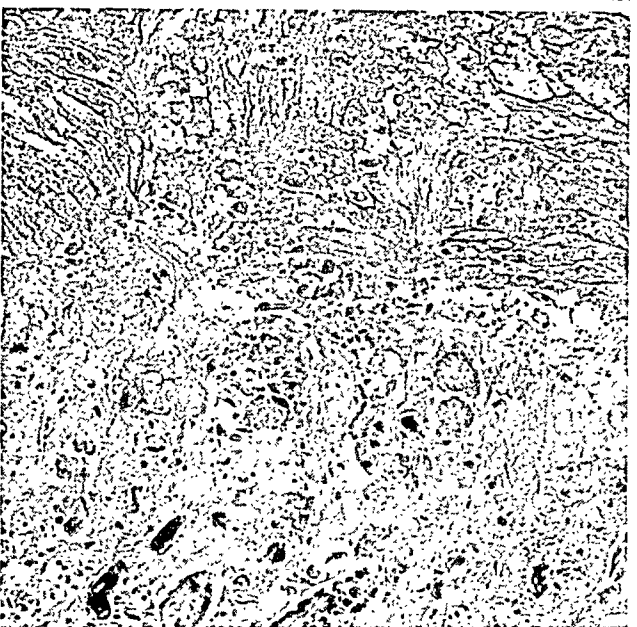
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Tullis

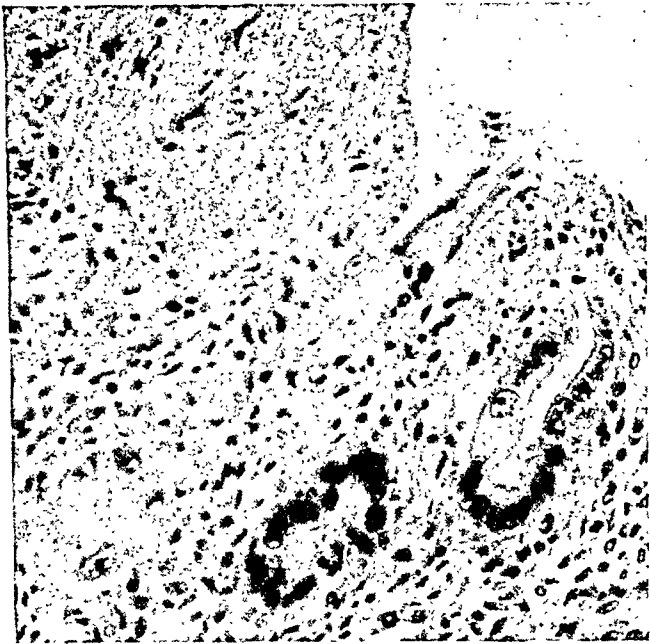
Total Body Irradiation

## PLATE 119

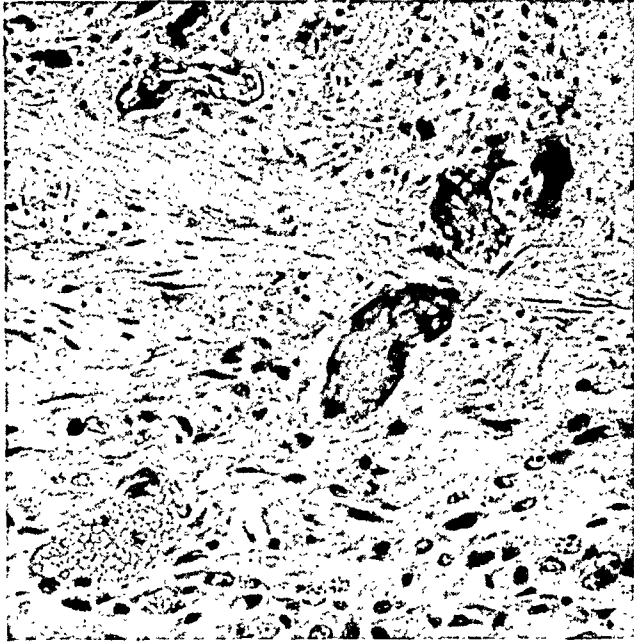
All photomicrographs—Figures 25 through 30—are taken from lesions in swine exposed to ionizing radiations from the atomic bomb tests at Bikini.

- FIG. 25. Small intestine of pig 419. This is at the junction of an ulcer and a less involved portion of mucosa. There is a sharp line of demarcation between the totally necrotic area and the area in which there is distortion, vacuolation, and pyknosis in the epithelium.  $\times 200$ .
- FIG. 26. Small intestine of pig 419. Fibrin thrombi and one agglutinative thrombus in vessels beneath the mucosa and ulcerated area seen in a portion of Figure 25.  $\times 200$ .
- FIG. 27. Fibrillar degeneration of connective tissue cells in the loose areolar tissue of the subserosa of the duodenum of pig 240. There is some extravasated blood and edema fluid. Degeneration of this type was seen in connective tissue elements in other parts of the body.  $\times 400$ .
- FIG. 28. Stomach mucosa of pig 419. There is marked variation in the cells making up the glands. Some are vacuolated, some pyknotic and some assume bizarre, distorted forms.  $\times 400$ .
- FIG. 29. Lungs of pig 337. Edema, fibrin, and scattered macrophages are found in the alveoli 12 days after irradiation. The vessels of the alveolar septa are congested. There is no inflammatory cellular infiltration.  $\times 300$ .
- FIG. 30. Lungs of pig 337. Hemorrhage in the alveoli and an agglutinative thrombus in an arteriole are seen in a different portion of the same lung shown in Figure 29.  $\times 300$ .

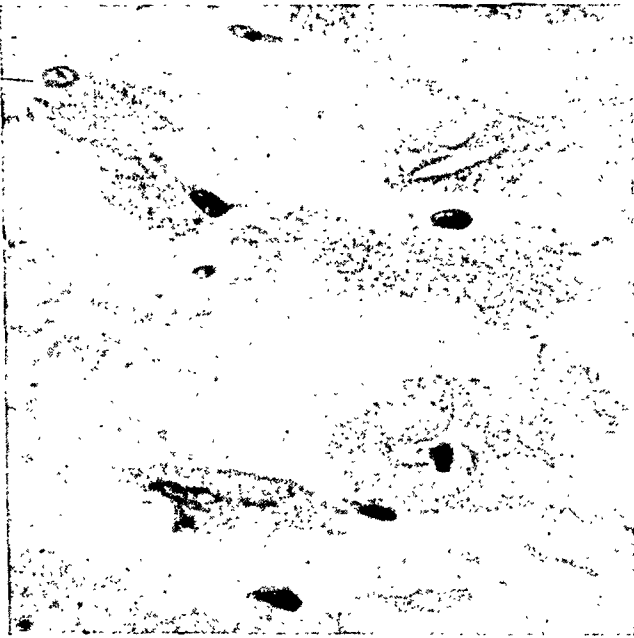
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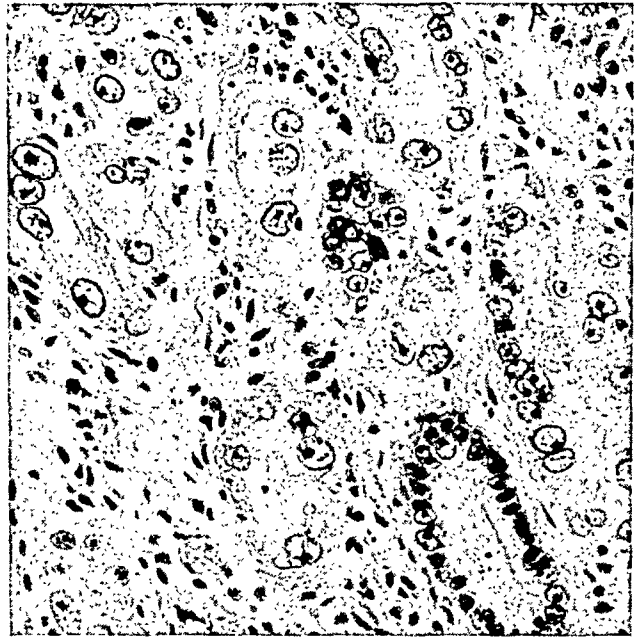
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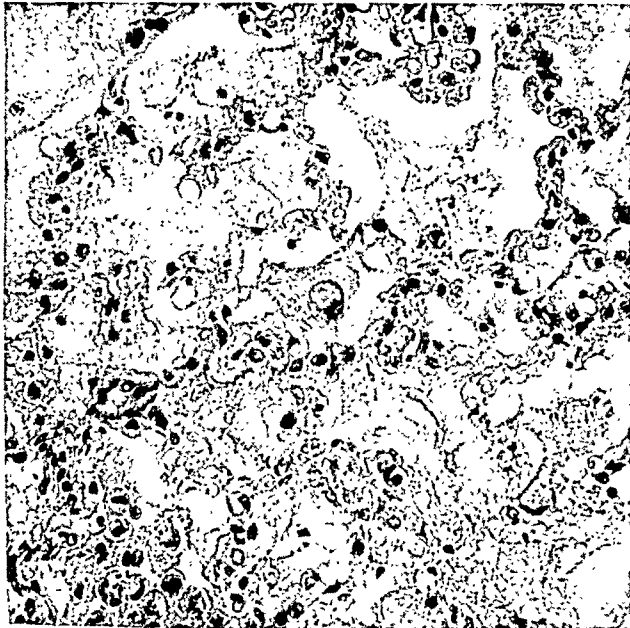
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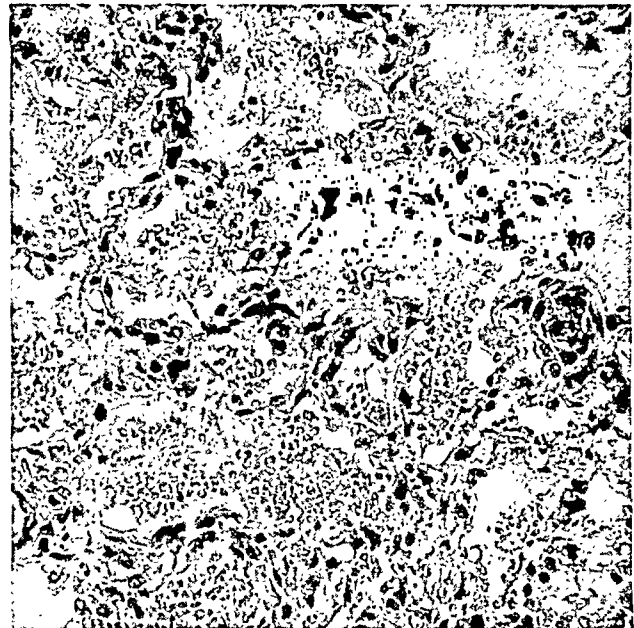
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Tullis

Total Body Irradiation

PLATE 120

All photomicrographs—Figures 31 through 36—are taken from lesions in swine exposed to ionizing radiations from the atomic bomb tests at Bikini.

FIG. 31. Kidney of pig 419. Small necrotic focus in tubular epithelium associated with hemorrhage of interlobular vessels. The surrounding tubules and two glomeruli appear normal.  $\times 100$ .

FIG. 32. Bladder of pig 364, 7 days after irradiation. The mucosal epithelium is degenerated and most of it has been sloughed off. The subepithelial tissue is edematous and one vessel is occluded with an agglutinative thrombus.  $\times 300$ .

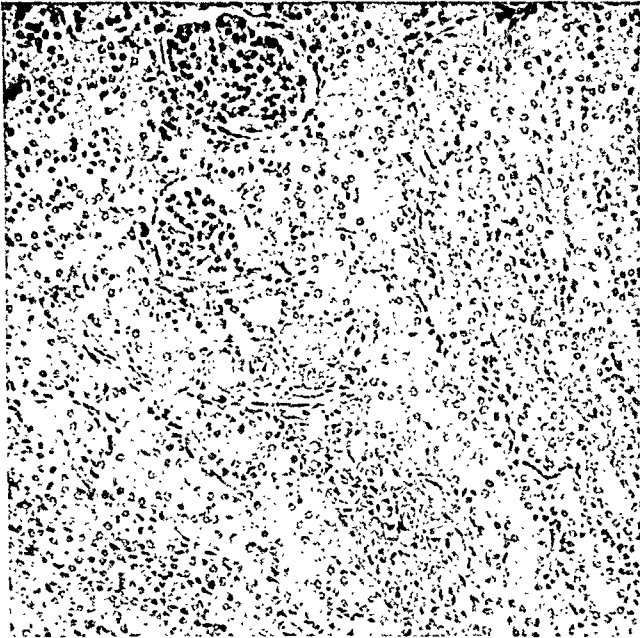
FIG. 33. Testis of an immature boar, pig 459, 5 days after irradiation. The spermatogonia are nearly all destroyed. The ghost outline of one or two of them may still be seen. The seminiferous epithelium is reduced to one cell layer composed chiefly of so-called indifferent cells.  $\times 400$ .

FIG. 34. Ovary of a sow, pig 364, approximately the same age as the boar shown in Figure 33, 7 days after irradiation. Most of the residual ova are atretic while others are vacuolated and appear to be degenerating. The stroma is edematous.  $\times 300$ .

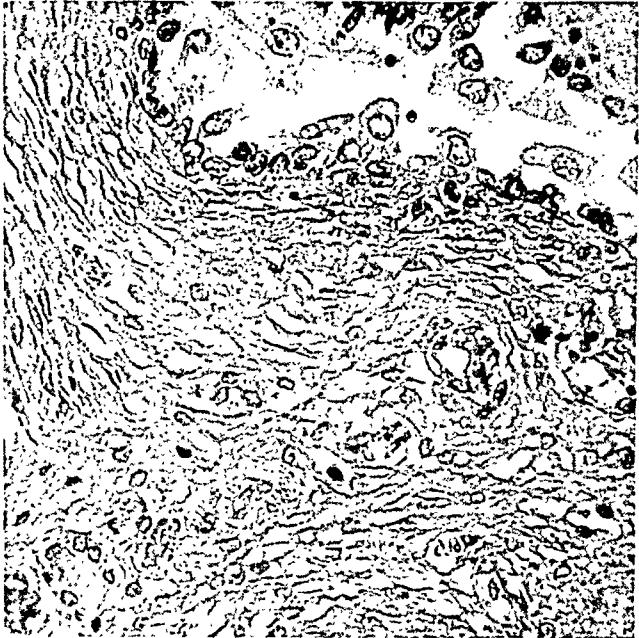
FIG. 35. Skin of ear of pig 265. Vacuolation of nuclei and clumping of chromatin are found in the epidermis, chiefly in the basal layer, 6 days after irradiation. The dermis is edematous and one vessel contains an agglutinative thrombus.  $\times 400$ .

FIG. 36. Hair follicle of pig 265. There is no clear definition of the several epithelial layers that envelop the root of the hair and many of the cells are vacuolated.  $\times 200$ .

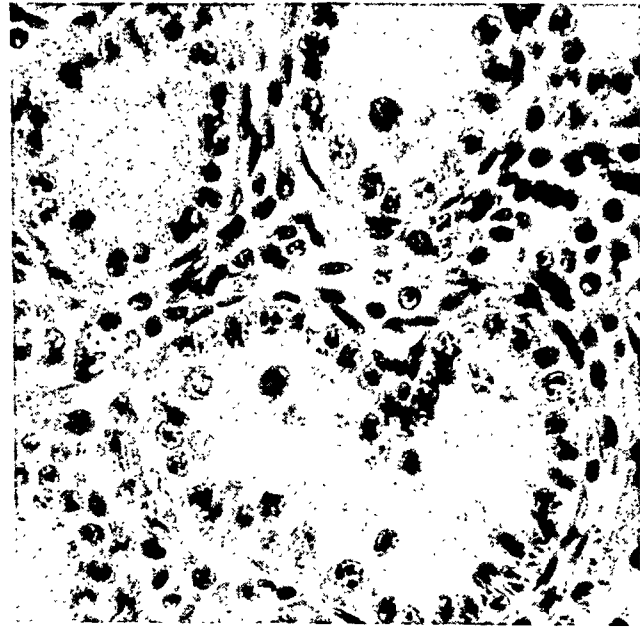
31



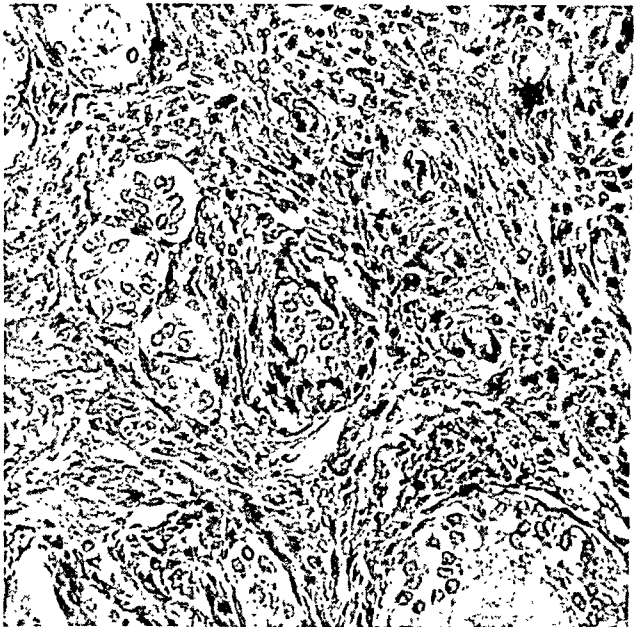
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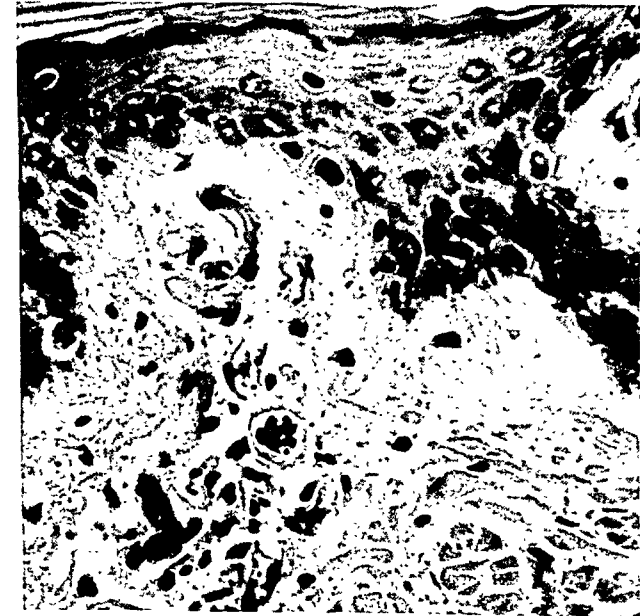
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Tullis

Total Body Irradiation





## PATHOLOGY OF ATOMIC BOMB CASUALTIES\*

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The energies released in atomic explosions embrace the entire range from that of mechanical shock waves to that of gamma rays. The effects on exposed tissues are modified not only by the characteristics of the bomb but by the conditions of exposure. As it happened, the injuries were not qualitatively different in Hiroshima and Nagasaki. The descriptions of the lesions must, however, be regarded as representative only of the changes produced at those two cities. Whether or not the explosion of other bombs, or of like bombs under different conditions, would produce comparable effects cannot be stated.

### METHODS AND MATERIALS

Since the Joint Commission was not able to begin its work in Japan until some 6 weeks after the bombings of August 6, 1945, at Hiroshima and August 9, 1945, at Nagasaki, by which time most of those who were to die from radiation effects had succumbed, it was necessary to secure clinical records and necropsy protocols and specimens from Japanese clinicians and pathologists to supplement materials obtained at necropsy by members of the Joint Commission itself. Some of these pathologists were attached to the Commission in a group of some sixty Japanese physicians who were to aid in the clinical field study. Others had been with "research parties" that had been sent into the stricken cities by various universities and by the Japanese Army Medical Service to conduct medical investigations. To the last mentioned we owe the only available records and materials from the patients dying within the first

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This paper is a summary of a portion of a report to the Surgeons General of the Armed Forces by the "Joint Commission for the Investigation of the Atomic Bomb in Japan." The Commission was comprised of U.S. Army personnel under the direction of Col. Ashley W. Oughterson, M.C., of personnel of the "Manhattan District" under Col. Stafford L. Warren, M.C., and of U.S. Navy personnel under Capt. Shields Warren, M.C., U.S.N.R. After the field study which was designed to obtain as detailed and accurate an estimate as possible of the types and numbers of casualties, of the mechanisms of injury, and of factors in protection, the report was prepared at the Army Institute of Pathology.

few days following the bombing. These were obtained for study through the aid of the Japanese Surgeon General Hiraye. Many of these "research parties" were staffed by the best available Japanese scientists who did work of a high order of excellence. Gradually a list of these pathologists was constructed—no small task since provincialism and professional jealousy were not unknown in the academic circles of Japan. In the work of establishing contact with these men and obtaining the necessary materials, the Joint Commission was greatly aided by Dr. Masao Tsuzuki, then Professor of Surgery at the Tokyo Imperial University, and chief of the medical section of the Japanese National Research Council. It was his influence that unlocked cabinets of material that could otherwise easily have remained sealed. The records were translated word by word from the original protocols by a team comprised of the pathologist who had performed the necropsy, one of the English speaking pathologists attached to the Joint Commission, and one of the American pathologists of the Commission. In many instances it was necessary to travel into remote parts of Japan to obtain the records and materials at first hand from the files of the smaller universities. When gross specimens were still available, they were checked against the descriptions in the protocols. Most of these were found to be quite accurate. Blocks were then taken from the original specimens to check and supplement the usually better fixed tissue in the smaller specimen jars and also the paraffin blocks and finished histologic preparations that were requested in the original letters written by Dr. Tsuzuki. The histologic slides were checked also against the protocols as they were received.

The records and materials were brought back to the United States where additional sections were prepared and illustrations made through the facilities of the Army Institute of Pathology. The Naval Medical Research Institute aided materially in working up the Nagasaki material. The necropsy protocols were transcribed into standard United States Army Medical Department form. Each was assigned a "Key Number," by which it is filed under the general A.I.P. accession number 158930. Reference to patients in the present text is made according to this key as "K" followed by the appropriate numeral.

After a preliminary review, tissues from 170 patients were found sufficiently well preserved to warrant detailed histologic study. For purposes of statistical tabulation, however, only the last 16 necropsies of the Nagasaki series, which had been performed after the 40th day by members of the Joint Commission itself, were found useful, since only in those protocols was there a record of the distance from the point of

explosion, and of possible protective factors. These factors usually were recorded in the Hiroshima protocols since the Japanese pathologists there had been keenly aware of their supreme significance. Moreover, many of the patients had been soldiers within well defined areas of the large military encampment (Chugoku Army Headquarters), one of whose boundaries was almost directly beneath the point of explosion of the bomb. Consequently, the detailed tabulations are based on a total of 110 patients, 94 from Hiroshima and 16 from Nagasaki, although occasional reference is made to the earlier Nagasaki material. It may be unequivocally stated that at least the temporal sequence of anatomic changes in heavily exposed patients in the two cities is similar. Lamentably, an exact comparison by distance from the bomb is not possible on the basis of the material at hand.

The concurrent clinical studies of some 14,000 patients, and the protection and casualty surveys are summarized in the general Medical Report of the Joint Commission.<sup>47</sup>

### THE INJURIOUS FACTORS—GENERAL STATEMENT

The physical factors of distance and the condition of the blanket of atmosphere at the moment of explosion are two important determinants of the effects of the bombs. Both bombs were exploded many hundreds of yards above the cities. In the discussion to follow the distances are stated from the point on the ground above which the explosion occurred, not from the explosion itself. The former point is variously referred to as the "hypocenter," "ground center," or "ground zero."

The injurious factors and the damage inflicted are summarized in

TABLE I  
*Energies and Effects of the Atomic Bomb*

Energy	Injury	Type or target of injury
I. Mechanical	A. Trauma	1. Direct (blast) 2. Indirect (falling débris)
II. Radiant		
a. Thermal	B. Burns	1. Direct ("flash burns") 2. Indirect
b. Ionizing radiations	C. Radiation effect	1. Skin 2. Gastro-intestinal tract 3. Gonads 4. Lymphoid tissue 5. Marrow 6. Other tissues

general terms in Table I. Often the three main types of injury coexisted: traumatic, thermal, and ionizing radiation. For purposes of clarity, these will, however, be discussed separately. Traumatic and thermal injury accounted for the vast majority of the casualties.

*Traumatic Injuries*

In considering the mechanical effects of the explosion, direct blast injury and the indirect effects produced by collapsing buildings and flying débris must be distinguished. As in the bombings of Great Britain, the latter was far more important in producing casualties than the former.

Direct blast injury to ears, lung, and intestines analogous to that inflicted by high explosive bursting within a few feet was almost unknown. At the prevailing distance from the target the explosion did not have the hammer-blow effect of high explosive,<sup>20,98</sup> but rather was like a sudden violent gust of air which lasted for a brief but appreciable period. There was a short, positive phase followed by a longer, but less destructive, negative phase. The relative ineffectiveness of the latter was indicated by the nature of the damage to buildings, most of which showed a preponderance of effects indicating a thrust away from the bomb. At a Hiroshima hospital soon after the bombing only 8 of 371 patients who were examined had ruptured ear drums, although 19 suffered temporary deafness. Seventy-six per cent of this group of men had been within 2000 yds. of the center. In the survivors examined by the Joint Commission after the sixth week, less than 1 per cent, even of those who had been within the first 1000 yds., had ruptured ear drums, and beyond 1500 yds. the incidence fell below 0.1 per cent. Not a single middle ear or tympanum was available for microscopic examination from the patients in whom there was a clinical suggestion of blast injury. The foci of pulmonary emphysema and atelectasis without hemorrhage observed in some of the early casualties (Fig. 20) are difficult to interpret. These were found frequently at death in patients who had not been exposed to blast. Many patients lost consciousness for brief periods, although they could not remember any direct trauma aside from blast. A few were injured by being hurled forcibly, sometimes for considerable distances, against solid objects, but this must be considered an indirect effect of the blast.

Immensely more injury was inflicted by the indirect effect of the blast—the falling débris and masonry. How many were killed outright will never be known accurately. Thousands were pinned beneath the wreckage and were soon consumed by the holocaust of fire that swept the city and which made rescue impossible. In October of 1945, 2 months after the explosion, it was remarkable to note how few were the survivors who had suffered really severe injury. Thus in survivors at Hiroshima the incidence of fractures was less than 4.5 per cent. It was not that injuries

were few; rather, almost none who had lost the capacity to move escaped the flames.

Very characteristic were the lesions inflicted by flying glass (Fig. 1). This material often split into long spear-shaped fragments, even as far as 3 miles from the explosion. The fragments were hurled with such force as to become embedded in the wood of the opposite walls. As was to be expected, the wounds were multiple and often penetrating. Often keloids appeared following injury of this type (as is not infrequent in the Japanese race), and arteriovenous aneurysm sometimes resulted from injuries of the vessels.

Since some of the patients had suffered injury also from ionizing radiations with consequent leukopenia, it is obvious that even minor traumatic wounds might become the seat of infections which could become generalized.

### *Burns*

As in the case of the mechanical forces, direct and indirect factors in the causation of the burns must be considered. Direct "flash" burns resulted from exposure to the radiant energy of the bomb. This energy was transmitted in a spectrum resembling that of the sun. In the present section only that band including the ultraviolet, visible light, and infra-red rays will be considered. The exact intensities of these various components cannot be stated, but it is probable that all contributed to the damage to the skin of exposed human beings. All of the component rays were transmitted in straight lines at the velocity of light. Consequently, only surfaces directly exposed were burned and intervening objects cast "shadows."

To understand the "flash burns" one must conceive of a very large amount of radiant energy acting for an extremely brief interval of time. The intensity is indicated by the effect on granite which within several hundred yards showed fragmentation of its surface caused by an unequal expansion of its components. It has been estimated that a temperature of at least 2000° C. is necessary to produce this effect. The brevity of the peak intensity of the flash is indicated by the fact that the "shadows" of constantly moving and easily shriveled objects, such as leaves, are sharply outlined on wood behind them—and exposed wood was superficially carbonized even at 3000 yds. from the center.<sup>47</sup>

The rays in their rectilinear course burned whatever profile, including that of man, directly faced the center of the explosion. Thus the burns typically were very sharply outlined. Intervening objects also cast sharp protective shadows on the skin. Those close to the bomb suffered searing

or charring of the skin to the level of the subcutaneous tissue and died shortly (Fig. 3). Death was the fate of more than 95 per cent of those directly exposed to the bomb within 1200 yds. of the point above which the bomb exploded. Beyond this, clothes were some protection. The darker shades absorbed more heat as is demonstrated by selective scorching of the dark polka dots, or stripes, or flowers on a contrasting lighter pattern. Occasionally, such parts of the skin as underlay the darker portions of the pattern were selectively injured (Fig. 2). Sometimes, however, burns occurred beneath unaltered cloth, where it was tightly stretched over the skin in those patients closest to the explosion (Fig. 4).

Between 1500 and 2000 yds. the burns varied in their severity, some showing complete destruction of the derma, others being of second degree. From the data of Ashe and Roberts<sup>5</sup> it is learned that a temperature of 400° C. acting for approximately 0.5 seconds is necessary to produce a second degree burn. The exact numbers of calories absorbed by various surfaces exposed to the bomb are not known, since the duration and intensity curve of the heat flux are matters of conjecture. As 2000 yds. was approached, some cutaneous surfaces ultimately showed, after an initial phase of erythema and blistering, depigmentation with superficial damage or no damage to the epithelium (Fig. 4). Beyond this distance, and up to 4000 yds., the opposite change, an intense pigmentation resembling that of ordinary but extreme sunburn, followed the initial erythema. This deep chocolate colored mask was well shown in a group of men who had been prisoners in the city jail at approximately 2500 yds. from the hypocenter (Fig. 5).

Closer inspection of the sharply outlined "profile" or "flash" burns after healing showed that even the most severely burned areas usually were surrounded by an intensely pigmented zone, and this, in turn, by a narrow band of depigmented tissue whose overlying epithelium was intact (Fig. 8). Even the centrally depigmented but otherwise little damaged skin showed similar phenomena of pigmentation—a broad pigmented area surrounding the central, most-directly exposed zone of depigmentation, with a narrow ring of depigmented tissue again separating the hyperpigmented skin from the surrounding normal tissue.

Practically all of the burns became infected, and many which had been of the second degree ultimately showed deep destruction of tissue as a result of bacterial action. The sharp outlines of the burns often were disturbed, as undermining ulcerations of bacterial causation developed. The infections, as in the case of the mechanical injuries, were particularly

important in those who were within 1500 yds. and who had received a significant dose of radiation.

Occasionally the thermal injury, coupled with infection, resulted in chondritis of the ear, which, in healing, produced a scarred, shrunk, and distorted auricle.

Keloids were observed frequently and were extreme in some patients (Fig. 7) within 10 weeks. Their subsequent development has been described by Block and Tsuzuki.<sup>9</sup> More than three-fourths of the healing second and third degree burns showed some overgrowth of scar tissue. The tentative conclusion of the Japanese observers was that keloids probably were not more frequent than would be expected in this race in ordinary, infected, poorly treated burns. This conclusion has not been altered by subsequent observations of the survivors.<sup>9</sup>

Histologically, there is evidence confirming the gross observation that depigmentation of the skin can occur even if the epithelium of the surface is not completely destroyed (Figs. 6, 11, and 12). In that portion of the skin which had been most severely burned, the epithelium of the surface was necrotic and contained pyknotic nuclear remnants, both of polymorphonuclear cells and large mononuclear phagocytes. Very few leukocytes were present in the adjacent corium and deeper lying perivascular tissue. At the margins of the denuded area the epithelial cells of the malpighian stratum had lost their pigment and no dermal melanophores were found. Some other epithelial cells had become vacuolated and were in disarray. At a still greater distance from the zone of destruction the epithelium was relatively well preserved and its basal layers had become excessively pigmented. There some dermal melanophores also were seen. More laterally still there was a much less pigmented zone but there again was evidence of a more deeply penetrating burn. The zone of hyperpigmentation corresponded to that observed grossly. In this particular case, however, there had occurred also some severe injury more peripherally which precluded complete interpretation of the gross appearance. These observations indicate that complete destruction of epithelium is unnecessary for depigmentation to occur. They suggest also that some special band of the spectrum, probably in the ultraviolet, might be responsible for this phenomenon. The diverse effects upon the skin of different bands in the ultraviolet have been pointed out recently.<sup>12,13</sup> Much is still to be learned concerning the action of high intensities in the ultraviolet range.

The results of infection in the presence of leukopenia were seen in a section from a small burn in a patient who was at 1000 yds. and who received no other serious burns. The epithelium had completely sloughed



(Fig. 13). On the homogeneous, dull pink-staining surface of the collagen were present irregular, granular, purple-staining masses of cocci. Some of these occurred in islets as much as 1 mm. below the surface and one clump actually lay within a bundle of smooth muscle (arrector pili). Squamous metaplasia had occurred in the ducts of the sweat glands. There appeared to have been actual proliferation of these cells, many of which were large and irregular with tremendous vesicular nuclei. The acini of the sweat glands, however, exhibited shrinkage of the epithelium and irregular thickening and compaction of the basement membrane, in some cases with partial collapse of the acinus. Nowhere was there a leukocytic infiltration in the vicinity of the bacterial masses. In one small portion of the surface a very thin layer of squamous epithelial cells remained. These cells were much swollen, some were multinucleated, and in all instances the vesicular nuclei contained very prominent chromatin knots. These changes are ascribed to thermal effect, but what direct rôle ionizing radiations played is difficult to assess. The evidence of indirect effect of the ionizing radiations lies in the total absence of leukocytic response to the injury and to the bacteria that are seen in section.

In another case, the subepithelial tissues contained a scanty exudate consisting largely of monocytes situated within extravasated fibrinous material. One vessel was found to have a necrotic wall and a lumen filled with a thrombus which consisted largely of fibrin (Fig. 14). Deeper in the subcutaneous tissue of the same specimen was a remarkable basophilic ground substance containing irregular large cells with basophilic cytoplasm. Some of these had the granules of mast cells (Fig. 15).

Severe burns caused by contact with flame rarely were observed by the Joint Commission, for the same reason that relatively few severe traumatic injuries were seen among the survivors. The gathering fires spread so rapidly within the cities that only those who were not severely injured could escape. Occasionally, dark clothing burst into flame on those within 1500 yds. of the hypocenter and contact burns resulted. Such burns lacked the sharp outlines produced by shadowing, and the "profile" characteristics seen in the flash burns. Their course also was affected by infection and leukopenia.

### *Radiation Effects*

#### *Etiologic Factors*

During the explosion of the atomic bomb, vast quantities of ionizing radiations were released in the form of gamma rays, neutrons, beta particles, alpha particles, radioactive fission products, and possibly some

of the unexploded radioactive substance of the bomb itself. Factors of distance and shielding are of special importance in considering the effects of these radiations. Thus, the beta and alpha particles can be disregarded since they travel only a short distance through air. The fission products largely were swept into the stratosphere by the violent updraft created by the heat of the explosion. There was no biologic evidence that significant amounts of these materials had been deposited upon the target. Potentially of greater significance may be the neutrons, for they can be projected for considerable distances through the atmosphere. Their effectiveness in damaging tissue is several times greater than that of gamma rays, measured in roentgens equivalent physical (rep).<sup>41</sup> The complexity of their interaction with tissue has been described by Zirkle.<sup>97</sup> Furthermore, they can induce radioactivity in various materials. While there is no evidence of a significant amount of induced radioactivity at the hypocenter (as attested by the absence of radiation effects in the Ishizuka military unit which had been sent to this area for rescue work after the explosion<sup>47</sup>), nevertheless, neutrons in their passage through the atmosphere produce a certain proportion of the highly penetrating gamma radiations. Since at any point there was a complex of radiations, the inclusive term "ionizing radiations" will be employed hereafter. There is justification for this in that no qualitatively different effects have been observed in the tissues upon exposure to the various types of radiations.<sup>10,79,90</sup> In each instance the damage inflicted is proportional to the quantity of energy absorbed, not to the quantity delivered.<sup>90</sup> It follows that the direct injury is localized to the sites where ionization occurs.<sup>70</sup>

The gamma rays merit special discussion, since they were probably by far the most important of the biologically damaging radiations emitted from the bomb at the instant of detonation and from the rapidly rising column of fission products. The higher the energy and the shorter the wavelength the greater is the penetrating power in tissue. Radiations of longer wavelength ("soft" gamma rays) tend to be absorbed in the superficial tissues, while some of those of shorter wavelengths may pass entirely through the body with little or no absorption. Thus the wavelength mixture as well as the distance from the explosion will determine the localization and extent of the greatest damage. During the explosion of the atomic bomb a spectrum of gamma rays of widely varying wavelengths was emitted. The proportions of the gamma ray energy of these various wavelengths in the radiations in Japan are not known. Moreover, the gamma rays are to some degree scattered by intervening materials, including air, thereby modifying the wavelength by the Comp-

ton effect,<sup>87</sup> with the result that very little is known about the quality of the radiations actually delivered to the body. Another factor of biologic importance is the dose rate. Within limits still to be determined, the more rapidly a given quantity of radiation is administered, the greater its injurious effect.<sup>21,41</sup> In the case of the atomic bomb the total duration of exposure was, at most, a few seconds, and probably by far the greatest dosage was delivered in a very small fraction of a second.

Although the exact nature, spectrum, and intensity of the ionizing radiations produced by the atomic bomb are incompletely known, their action upon the tissues is analogous to that of ordinary x-rays. The most striking changes were found in the skin, gastro-intestinal tract, testes, lymphatic system, and bone marrow. As a consequence of the direct damage to marrow, there resulted all of the tissue changes characteristic of aplastic anemia and of the associated infection. Thus, again, direct and indirect effects were manifest.

As to the range of the radiation effects, it may be stated that severe evidences of radiation injury were rare beyond 1500 yds. from the hypocenter, if they existed at all.

### Clinical Classification

Patients with radiation injury fall into four major groups which will receive brief discussion. The clinical observations are presented in detail in the body of the Medical Report.<sup>47</sup>

Group I: Patients dying in the first and second weeks.

Group II: Patients suffering severe symptoms or dying in the third, fourth, fifth, and sixth weeks.

Group III: Patients dying after the sixth week.

Group IV: Mild cases.

*Group I.* The factors responsible for death during the first 2 weeks may be: (1) An unusually high dosage of ionizing radiations either because of proximity to the bomb, or because of lack of efficient shielding. (2) An unusual susceptibility to ionizing radiations. Individual as well as species variations in susceptibility have often been observed.<sup>38</sup> (3) Concomitant severe thermal or traumatic injuries, or intercurrent infections, or causes of death not directly connected with the bombing. Multiple injuries were the rule.

With the exception of this last sub-group, which will be ignored for the present, many patients suffered during the first few days from a fatal illness that to the Japanese medical observers at first seemed mysterious,

until it was realized that they were dealing with the syndrome of "radiation sickness." The signs and symptoms in general resembled those found in animals after exposure to massive doses of ionizing radiations,<sup>69</sup> and probably represented largely the direct effects of these radiations, complicated in some instances by infection.

Patients complained of nausea and vomiting, often within  $\frac{1}{2}$  hour of the bombing, or during the succeeding hours or days. This was followed shortly by anorexia, malaise, severe diarrhea that in a few instances became sanguineous, intense thirst, and fever that ascended step-wise day by day. Death ensued in coma or delirium within the first 2 weeks. Leukopenia, especially lymphopenia, was found as early as the first day after the bombing in some of these patients, but others had no leukopenia at the time of death as late as 7 days after the bombing. Thrombocytopenia and an increasingly severe anemia appeared in that order, but usually not until after the second week.

In this early group there was already histologic evidence of radiation effect upon the hair follicles, gastro-intestinal tract, lymphoid tissues, bone marrow, and gonads, but neither epilation nor purpura had become clinically manifest in the vast majority of patients.

*Group II (Patients Dying During the Third, Fourth, Fifth, and Sixth Weeks, or Surviving Severe Clinical Symptoms).* In group II there were not only the clinical evidences of the direct damage by ionizing radiations such as epilation, but also the manifestations of aplastic anemia consequent upon destruction of the bone marrow. Bacterial infection, an indirect result of the radiation, was, as usual, responsible for the necrotizing lesions characteristic of the aplastic anemia. Purpura was now a frequent sign. Several factors were probably concerned in the pathogenesis of this condition: at first, probably the appearance in the blood of a substance with the properties of heparin<sup>4</sup>; later, thrombocytopenia which often reached levels below 10,000 per cmm.; and possibly also infection and vitamin deficiency with their effect on capillary fragility.

The usual sequence of events was as follows: Nausea and vomiting on the day of the bombing were the first evidences of the disease, followed by any or all of the symptoms characteristic of group I, but in milder form and persisting for only a few days. There was then a complete remission, until the appearance of epilation some 2 weeks after the bombing. Approximately 5 days after this, accompanied by increasing malaise, there was a daily ascending unremitting fever. At approximately the same time pharyngeal pain might appear or it came somewhat later. Petechiae and ulcerative lesions of the skin, lips, mouth, and pharynx became manifest within a few days after onset of the febrile episode.

Sanguineous diarrhea, associated with an ulcerative gastritis and enteritis, was frequently a prominent symptom at the height of the disease and sometimes appeared very early, as previously described. Leukocytes and platelets reached very low levels at the time of the fever and there was often severe anemia. The patient usually died within 1 or 2 weeks of the onset of the fever, approximately 1 month after the bombing.

Some patients exhibiting all or several of the symptoms of purpura, gingivitis, and severe or even necrotizing pharyngitis survived after a febrile period. Pharyngitis ceased before, petechiae before or during, and oropharyngeal lesions usually after the end of the febrile period. Recovery was associated with an increase in the circulating leukocytes and platelets. Many of these patients remained in an anemic and generally debilitated condition for long periods. In survivors the red blood cell count tended to fall slowly for some weeks after the end of the severe illness.

*Group III (Patients Dying after the Sixth Week).* In some members of group III in whom the bone marrow failed to recover or exhibited a maturation defect, the symptoms previously described continued and the patients died after a chronic illness. Patients with severe symptoms who survived beyond the sixth week, but who ultimately succumbed, usually were emaciated.

More commonly, with late fatality, the marrow tended to recover toward the end of the sixth week, and concomitantly most of the striking manifestations of the aplastic anemia, such as purpura, disappeared, but the patient nevertheless died of pneumonitis or enteritis—either a new illness of sudden onset, or an exacerbation of a smoldering lesion that had its inception during the earlier leukopenic phase. In these patients there was now a leukocytosis and the lesions contained an abundance of polymorphonuclear cells. There was usually no longer a marked thrombocytopenia, but anemia often was profound.

*Group IV (Mild Cases).* Persons who were situated near the limit of the range of the radiation, or who, although close to the center, were shielded by heavy buildings, manifested mild effects. In some, who were otherwise completely asymptomatic, leukopenia was discovered during a routine blood count. Some merely complained of anorexia and malaise. Diarrhea was a common complaint. Others had mild or even severe epilation without other symptoms. When, however, purpura or oropharyngeal lesions appeared, the patients usually also had epilation and passed through a febrile illness of varying degrees of severity such as was described in its most serious form in group II.

Most patients with the milder symptoms recovered completely. There were some, however, who had not regained their feeling of well being even 3 months after the bombing. A few of these people had a persistent leukopenia of approximately 3,000 at the time that the Joint Commission left Hiroshima (January, 1946). A moderate anemia was more common. What rôle dietary factors played in the anemia is not clear nor is there any information concerning how long the depression of the bone marrow could persist.

The important symptoms of the severest cases in the various groups are epitomized in Table II, and the anatomic findings are summarized in Table III.

TABLE II  
*Clinical Manifestations of Severe "Radiation Effect"*

Manifestation	Group		
	1st and 2nd weeks	3rd to 6th weeks	After 6th week
Epilation	o	+	+
Petechiae	o	+	o
Necrotic gingivitis and oropharyngitis	o	+	o
Diarrhea	+	+*	+†
Pneumonitis	o	+*	+†
Leukopenia	+	+	o

\* Lesions usually do not contain polymorphonuclear leukocytes.

† Lesions usually contain polymorphonuclear leukocytes.

Nausea and vomiting, occurring soon after the bombing, were frequent among those who later showed other evidences of radiation effect.

Fever was a common finding in all groups before death.

### SYSTEMATIC SURVEY OF THE LESIONS

In the preliminary clinical survey it has been brought out that the character of the lesions was dependent on three important factors: the date of death, shielding and distance from the bomb, and the presence of severe thermal or mechanical injury.

The possible significance of the interval between radiation injury and death in relation to dosage and susceptibility has already been considered. It is among patients dying in the first 2 weeks that the direct radiation effects as seen histologically are least obscured by the infections that are consequent upon aplastic anemia. During the month following this initial period the lesions are those of aplastic anemia of any cause together with some persistent identifiable direct radiation effects. The hemorrhages observed during this interval and for some time beyond may have a complex pathogenesis as will be outlined. After the sixth week most deaths can be considered accidents of overwhelming respiratory or enteric infection, since the bone marrow usually has recovered by this time, although it remained aplastic in occasional patients.

TABLE III  
Important Anatomic Changes in Severe "Radiation Effect"

Tissue	Important Anatomic Changes in Severe "Radiation Effect"		
	Group I Patients dying in weeks 1 and 2	Group II* Patients dying in weeks 3, 4, 5, 6	Group III† Patients dying after week 6
Adipose tissue	Usually no depletion	Occasionally depletion	Usually depletion or organizing
Lung	Occasional hemorrhage and edema	Necrosis and hemorrhage	Focal necrotizing or organizing pneumonitis
Bone marrow	A. Hypoplasia	Usually A. Hypoplasia Occasionally B. Marked reticulum hyperplasia C. Focal myeloid regeneration D. Marked myeloid hyperplasia	Usually Focal myeloid regeneration C. Marked myeloid hyperplasia D. Marked myeloid hyperplasia Occasionally A. Hypoplasia B. Marked reticulum hyperplasia As in group II, and occasionally regeneration of lymphoid tissue Necrosis and ulceration
Lymph nodes and spleen	Extreme decrease of small lymphocytes	As in group I, and atypical mononuclear cells	Focal necrosis and ulceration
Gastro-intestinal tract	Atypical mitotic figures and epithelial cells	Necrosis, hemorrhage, and ulceration	Focal necrosis of hair follicles; usually no other changes
Neck organs	Atypical mitotic figures and epithelial cells	Necrosis, hemorrhage, and ulceration	Regeneration of hair follicles; usually no other changes
Skin	Unknown	Necrosis, hemorrhage, and ulceration	Extreme atrophy
Gonads (especially testis)	Incipient atrophy	Petechiae and necrosis, atrophy of hair follicles Severe atrophy	Extreme atrophy

These are discussed in the text.

\* No polymorphonuclear cells in lesions.

† Polymorphonuclear cells in lesions.

No distinction is made in this table between the direct effects of ionizing radiations and the indirect effects resulting from infection, etc.

the text.

In others, despite hyperplasia of the marrow, the peripheral blood may continue to show aplastic anemia.

In dealing with radiation, shielding and distance factors are of prime importance. The range of effects was investigated in a large-scale clinical study in which both symptomatic and laboratory evidence was evaluated.<sup>47</sup> With rare exceptions, severe effects were confined to a radius of 1500 yds. about the hypocenter. The exceptions may represent inaccuracies of geographic localization, or possibly persons with an unusual susceptibility to radiation. Inaccuracies are inevitable when dealing with large numbers of persons or records, especially since some of the patients were gravely ill or unconscious and the history was obtained indirectly.

Patients with severe burns or mechanical injuries were almost never examined post mortem by the Japanese during the emergency, unless there was also evidence of radiation effect. The factors of severe burns or trauma must, however, be considered in classifying the cases since they may, by virtue of concomitant shock, infection, and other mechanisms, have contributed to the pathogenesis of various lesions found at necropsy. A "severe burn" was considered to be any of the following: (1) a first degree burn involving more than 20 per cent of the area of the body, (2) a second degree burn involving more than 10 per cent of the surface, (3) a third degree burn involving more than 2 per cent of the surface.

Since the purpose of the systematic survey is to present the pathologic data objectively, a classification of the cases has been made according to three major factors, as follows:

- A. By date of death
  - 1. Within the first 14 days
  - 2. Between the 15th and 42nd day
  - 3. After the sixth week
- B. By distance
  - 1. Within 1500 yds.
  - 2. Beyond 1500 yds.
- C. By the occurrence of severe burns
  - 1. Without severe burns
  - 2. With severe burns

To simplify the systematic description of the material, a 3-digit numerical classification will be employed, one digit being drawn from each of the three major classifications as outlined above and in the order in which they have been presented. Thus the first digit refers to date of



death according to the three subheadings under that classification, the second refers to distance under the two categories that have been established, and the third according to whether or not the patient had suffered severe burns. Thus, "212" indicates a patient who died between 15 and 42 days after the bombing, who was within 1500 yds. of the center, and who had severe burns.

The cases that came under study are classified in Table IV according to the scheme just outlined. It is apparent that the 16 Nagasaki cases whose distance from the bomb is recorded are all in group III. The others will be used only for general comparison with the Hiroshima material in relation to time of death.

TABLE IV  
*Number and Types of Cases from Which Necropsy Specimens Are Available*

Number of cases (Hiroshima)	Types of cases			Number of cases (Nagasaki)
	Identifying class number	Presence of burns	Distance	
Group I (Dead before the 15th day)				
3*	111	No burns	Within 1500 yds.	1
8	112	Burns		
1	122	Burns	Beyond 1500 yds.	
Group II (Dead 15th to 42nd days)				
58	211	No burns	Within 1500 yds.	52
5	212	Burns		
2	222	Burns	Beyond 1500 yds.	
Group III (Dead after the 42nd day)				
9	311	No burns	Within 1500 yds.	6
1	312	Burns		3
4	321	No burns	Beyond 1500 yds.	1
3	322	Burns		6
Data not given				7
94	Grand total			76

\* Material from additional Hiroshima cases, presumably in sub-group 112, although the distance was not recorded, is employed for illustrating certain lesions.

Certain gaps resulting from the fragmentary nature of some of the material will be evident in the attempted reconstruction of the sequence of events in the various systems. Obviously the reconstruction, based

TABLE V  
Hiroshima, Group I: Clinical Data on Patients Dying before the 15th Day

Autopsy K. no.	Day of death	Age	Sex	Distance (yds.)	Burns	Lacerations and contusions	Nausea or vomiting related to bombing	Diarrhea	Other signs or symptoms	White blood cells	Albuminuria	Fever
Sub-group III												
2	4	24	M	800	±	++	+	o	Weakness, excitement, then coma Headaches, simple fracture of femur	7500 (7)	+	++?
5	6	39	M	1000	±	++	+	o				++?
9	8	25	M	1500	±	++						++?
Sub-group II2												
1	3	13	M	1300	++		+	+	Sleeplessness "Cerebral symptoms" Simple fracture of femur	9500 (7)		+
3	5	15	M	1000	++							++
4	6	32	M	1000	++		o	++				++
6	6	13	F	1500	++		+	++		++		
7	7	34	M	1000	+		o	++		++?		
8	8	29	M	1200	++	+	+	o		++		
11	8	25	M	1200	++		o			5500 (7)	+	++
12	9	33	M	700	++		+	+				++?
Sub-group I22												
10	9	24	F	2000	++			+	Excited mental state, then coma	9500 (8)	+	++

No notation = no information given in record.

o = sign or symptom was stated to have been absent.

Burns: ±, minor burns; +, ++, ++++, severe burns.

White blood cells: Figure in parentheses indicates day on which stated count was made.

Fever: ++, fever present but temperature unstated; +, fever to 39° C.; ++, fever to 40° C.; ++++, fever to 41° C.

as it was on surviving material and recorded information, had certain archeologic aspects. Since the deficiencies in the records of the early cases are particularly lamentable, a special effort is made to present them in all available clinical and anatomic detail.

*Group I: Patients Dying within the First 14 Days*

In group I there already were profound evidences of radiation effects histologically, but epilation, purpura, and many of the spectacular indirect effects of aplastic anemia that are occasioned by the associated infections had not yet appeared. For this reason the deaths were at first clinically obscure.

The specific clinical data in the 12 patients in group I who were necropsied are recorded in Table V. There was no evidence of leukopenia at the end of the first week in the 3 patients for whom counts are available and who were stated to have been within 1500 yds. of the hypocenter. In many others, however, severe leukopenia had already

TABLE VI  
*Hiroshima, Group I: Heart*

Observations	Sub-group		
	III	II2	I22
Gross specimens available	2	8	I
Histologic specimens available	2	8	I
Epicardial hemorrhages	2	6	
Perivascular edema	I	2	I
Thrombus in venule		I	
Plasmacytic or mononuclear cell infiltration of myocardium		I	
Plasmacytic or mononuclear cell infiltration of endocardium		I	

developed in the first few days. The actual count may have been masked by severe dehydration. Differential counts are not available for these 3 patients.

The 3 patients of sub-group III, in whom both traumatic and thermal injuries were slight, are of particular interest since they seem to have succumbed purely to the effects of radiation. They were no closer to the bomb than many others who survived. Their only protection was the wood and pantile of the Japanese buildings in which they were at the time of the explosion. The mechanism of death will be considered in the general discussion that follows the systematic descriptions.

*Heart (Table VI).* Petechiae of the epicardium were commonly found grossly. Microscopically, there occasionally was evidence of edema about the vessels of the myocardium. In one patient, who had been severely burned, there was plasma cell infiltration just beneath the

lining cells of the endocardium, and focal necrosis of the myocardium with mononuclear infiltration of the myolemma. Such changes cannot be considered as specific radiation effects, since they have been described in patients with many kinds of infection<sup>31</sup> and with burns.

*Lungs (Table VII).* In most instances the pulmonary changes were those of focal atelectasis and emphysema, and edema of the interstitial tissues (Fig. 20). There was no certain evidence of blast effect, although hemorrhages were observed in one instance. Among the 8 patients of sub-group 112 there was one with "neutropenic pneumonia"—focal necrosis and hemorrhage without polymorphonuclear infiltration—and another with focal pneumonia of the usual type with a heavy polymorphonuclear leukocytic exudate. In the latter instance the marrow

TABLE VII  
*Hiroshima, Group I: Lungs*

Observations	Sub-group		
	111	112	122
Histologic specimens available	3	8	1
Focal atelectasis and emphysema	1	6	1
Edema of interstitial tissue	2	6	1
Edema fluid in alveoli	1		
Thrombi or emboli in pulmonary arterioles	1	1	
Focal parenchymal hemorrhages	1		
Focal "neutropenic pneumonia"		1	
Focal necrotizing pneumonia with polymorphonuclear cells		1	1
Additional observations			
Disseminated fibrocaceous nodules			1
Fibrous pleural adhesions		3	1
Ghon's complex		1	
Apical fibrous scar		1	

was hyperplastic although the patient (K-12) was stated to have been 700 yds. from the hypocenter. The lymphoid tissue of the lungs partook of the general atrophy which will be described. Hemorrhages tended to be more numerous in patients dying after the second week.

*Hemopoietic Organs in General.* In the first few days in heavily irradiated patients there was a very rapid disappearance of lymphocytes and myeloid elements, followed by proliferation of atypical cells. Some of these resembled plasma cells and others, of larger size, were intermediate in structure between the plasma cells and the large elements of the reticulum. There appeared also huge cells with irregularly folded nuclei resembling Reed-Sternberg cells.

*Spleen (Table VIII).* The weight of the spleen usually was less than 100 gm. In general, the capsule was wrinkled and the organ soft. The cut surface was dark red and pulpy, but little material was yielded upon

scraping. Malpighian corpuscles were invisible to the naked eye, but the trabeculae were distinct.

Microscopically, the reduction in size was accounted for by the fact that the lymphocytes had almost completely disappeared. Malpighian corpuscles were only vaguely outlined by the whorled structure of the

TABLE VIII  
*Hiroshima, Group I: Spleen*

Observations	Sub-group		
	111	112	122
Histologic specimens available	3	8	1
Weight less than 100 gm.	1 of 2*	4 of 6*	1
Rupture		1	
Decrease of lymphocytes	3	5	1
Absence of germinal centers	3	5	1
Atypical mononuclear cells	3	6	1
Hyaline change in vessels	3		
Heavy mature plasma cell infiltration		1	
Perifollicular hemorrhage		1	
Numerous eosinophils		1	

\* The weights of the other spleens were not recorded.

collagenous and reticular tissue that appeared as a collapsed network about the central arterioles (Figs. 28 and 29). The subendothelial tissue of the latter contained a brightly acidophilic, refractile material. The endothelium itself was well preserved. In the immediate vicinity of these central vessels were found only a few small mononuclear cells, some of which had the structure of plasma cells. The nuclei of some of the latter as well as those of certain larger cells, presumably of the germinal center, were in process of lysis. The cytoplasm of such cells had shrunk into an opaque granular mass. Among these cells there were many apparently empty spaces. The process thus appeared to be one of necrobiosis and there was little evidence of phagocytosis of lymphoid elements. However, erythrophagocytosis and hemosiderosis of moderate degree were in evidence in all of these spleens (Fig. 31). Perifollicular and intra-follicular hemorrhages were noted in one instance (Fig. 30). There was no apparent change in the fibro-elastic structure of the capsule and trabeculae. At the insertions of the latter upon the former there were deep indentations of the surface.

In most patients, despite the atrophy of the lymphoid tissue, there was proliferation of atypical cells (Figs. 30 to 32, and 40). This was already in evidence, in the earliest available specimen, on the third day after the bombing and might become so massive as to increase the weight of the spleen. The atypical cells were irregular in shape and size, and possessed neutrophilic or basophilic cytoplasm and generally hyper-

chromatic nuclei with thick nuclear membranes and prominent nucleoli. Some resembled reticulum cells, and others lymphoblasts or the "splenic tumor cells" described by Rich, Lewis, and Wintrobe.<sup>72</sup> Some cells were so bizarre as to suggest the Reed-Sternberg cells of Hodgkin's disease (Fig. 32). Many cells were in mitosis and some of the mitotic figures were multipolar or otherwise atypical.

In one spleen of sub-group 112, there were numerous mature plasma cells and large spindle-shaped or polygonal elements containing prominent eosinophilic granules. The pathogenesis of this change is unknown. In another patient of this sub-group, there was rupture of the spleen with a slight quantity of blood in the peritoneal cavity. This patient had sustained trauma to the head, and probably the splenic lesion was also of traumatic origin.

*Lymph Nodes (Table IX).* The essential changes in the lymph nodes resembled those seen in the spleen. Germinal centers were not seen and

TABLE IX  
*Hiroshima, Group I: Lymph Nodes*

Observations	Sub-group		
	111	112	122
Histologic specimens available	0	4	0
Decrease of lymphocytes		3	
Absence of germinal centers		3	
Atypical mononuclear cells		2	

there was a striking reduction of the numbers of small lymphocytes. Thus little more than the reticular skeleton of the node remained. This compaction of the reticulum cells was clearly evident as early as the fifth day, and was particularly striking in a node from a patient who died on the tenth day (Fig. 44). Numerous mast cells were found among the reticulum cells. At 10 days a few large cells with the structure of lymphoblasts and some bizarre mononuclear elements resembling the atypical cells of the spleen had appeared. An occasional large cell was found in mitosis. These proliferative changes became more prominent in time, as will be evident from study of the nodes in group II.

*Bone Marrow (Table X).* Disappearance of the myeloid tissue and the presence of atypical cells within the first week after the bombing is indicated by the study of aspirated vertebral marrow,<sup>43,52</sup> carried out by the Japanese and later reviewed by us, in patients who may be assumed to have received a considerable dose of radiation. The failure of delivery of leukocytes to the peripheral blood was confirmed by their absence in exudates in obviously infected lesions seen at necropsy.

TABLE X  
*Hiroshima, Group I: Bone Marrow*

Observations	Sub-group		
	111	112	122
Long bones			
Histologic specimens available	1	3	1
Hypoplasia	1	1	
Marked myeloid hyperplasia		2	1
Flat bones			
Histologic specimens available	0	2*	0
Hypoplasia		1	
Marked focal reticulum hyperplasia		1	

\* Distance is not known in these 2 patients. Since the histologic changes in the tissues are so striking an indication of radiation effect, these patients are assumed to have been within 1500 yds.

The earliest histologic sections of marrow came from patients dying on the sixth day. Unfortunately, these were derived from long bones, where active hemopoiesis is usually not in progress in the adult. Even in these marrows there already was evidence of proliferation of the reticulum and of the formation of plasma-like cells, a process which became increasingly prominent for a time after the second week. Thus in the marrow derived from the femur or humerus of a 39-year-old man who died on the sixth day, there were small amounts of cellular tissue among the fat cells, and widely dilated blood vessels (Fig. 35). Many of the cells resembled plasma cells. In the hematoxylin and eosin preparations they had wine-colored, deeply staining cytoplasm and pyknotic nuclei. There also were other elements, intermediate in size between them and the large stellate elements of the reticulum. Some of these still retained the phagocytic property since the cytoplasm was filled with erythrocytes. Occasionally a cell resembling a reticulum cell was found to have an enormous amount of cytoplasm and a much folded vesicular nucleus. These cells were not nearly so large as the megakaryocytes and the cytoplasm was more basophilic. Only rarely was a myelocyte found at this time. There was nothing that suggested well-functioning hemopoietic tissue. Evidence of the functional insufficiency of the marrow in this case was the total absence of leukocytes in a small portion of burned skin (Fig. 13).

The first specimen of marrow from a flat bone, the sternum, was from a patient of sub-group 112, dead on the tenth day (Fig. 58), whose distance from the bomb, unfortunately, is unknown. Typical myeloid tissue was not in evidence. In its place there were numerous plasma cells and some larger atypical cells which often were found in long strings, applied adventitiously to the thin-walled vessels of the marrow.

Another patient (Fig. 59), who died on the twelfth day and whose distance from the bomb is again unknown, exhibited in sections of sternum a much more striking focal hyperplasia of reticulum together with numerous plasmacytoid cells. This type of response, termed "marked focal reticulum hyperplasia," is discussed in group II and its description will not be further elaborated here.

In 2 patients, K-11 and K-12, stated to have been at 1200 and 700 yds., respectively, and who had sustained severe burns, there was myeloid hyperplasia in the long bones with "shift to the left." Granting the position of these patients to have been correctly stated, this occurrence represents an individual variation in response. Had there been no burns these patients might have been among the survivors.

*Gastro-intestinal Tract (Table XI).* The changes observed in the gastro-intestinal tract during the first 2 weeks included the occasional appearance, by the fourth day, in the earliest available material, of atypical epithelial cells and petechiae, and after the seventh day of occasional ulcerative lesions.

TABLE XI  
*Hiroshima, Group I: Gastro-intestinal Tract*

Observations	Sub-group		
	111	112	122
<b>Stomach</b>			
Gross specimens available	3	8	1
Histologic specimens available	1	7	0
Petechiae	2	3	1
Acute ulcer		1*	
Plasma cell infiltration	1	1	
Atypical epithelial cells	1		
<b>Small intestine</b>			
Gross specimens available	2	8	1
Histologic specimens available	2	5	1
Ulcers and hemorrhage		1*	
Atypical epithelial cells and mitotic figures	2†	1	
Petechiae			1
<b>Large intestine</b>			
Gross specimens available	1	8	0
Superficial ulcers	1*	1*	
<b>Additional observations</b>			
Ascariasis	2	3	

\* Gross only.

† There was also a Nagasaki case, K-175, dying on the 11th day, with similar changes in the epithelium, together with ulcers of the intestines.

Epithelial changes were demonstrated in a section of ileum from a patient who died on the sixth day. This material was well fixed soon after



death. The epithelium of the surface had in part sloughed, but without loss of substance of the underlying connective tissue. That which remained was extremely irregular in size and shape. Many epithelial cells were spindle-shaped and some possessed vesicular nuclei with large clumps of chromatin and irregular masses of basophilic cytoplasm (Fig. 80). Few small lymphocytes were left in the mucosa and there were relatively large numbers of plasma cells together with reticulum cells. The mucosa was edematous. The changes in the intestine of K-2, who died on the fourth day, were similar; a tripolar mitotic figure is illustrated in Figure 81. Such epithelial changes were observed in the small intestine of 3 patients, and once in the stomach. They closely resemble lesions found in animals experimentally exposed to gamma rays.<sup>27</sup> Either these atypical epithelial cells are not long viable, or else patients receiving a dose of radiation large enough to produce them do not live long, since they were not observed in patients necropsied after the second week.

Ulcerative lesions were not observed before the seventh day. In one patient at Nagasaki who died on the eleventh day, both bizarre, occasionally binucleated epithelial cells and focal necrosis of the mucosa and submucosa were observed (Figs. 82 and 83). Numerous bacterial masses were found in the tissue, but there were no leukocytes. Both the direct effects of ionizing radiation and the absence of the leukocytic defense mechanism may have contributed to the development of such ulcerative lesions.

Atrophy of lymphoid tissue was found in all instances in which there had been exposure to radiation. Heavy plasma cell infiltrations, however, were found in several instances. The essentially similar changes in the pharynx and tongue are described on page 880.

*Pancreas.* No significant changes were found in the pancreas.

*Liver (Table XII).* The liver was of the usual size. Microscopically, the cells appeared finely granular and displayed no fatty changes. The

TABLE XII  
*Hiroshima, Group I: Liver*

Observations	Sub-group		
	111	112	122
Histologic specimens available	3	8	1
Giant nuclei in pericentral hepatic cells	3	1	
Central congestion, slight	3	8	1
Edema of pericentral connective tissue	3		
Heavy plasma cell infiltration of portal zones		1	

nuclei of some of the cells near the central vein were large and hyperchromic. The central parts of the hepatic cell cords were narrower and the sinusoids there were more widely dilated than elsewhere. The walls

of many central veins appeared to be thick and to consist of loosely arranged collagenous material which extended outwards in strands among the cell cords (Fig. 93). This change is interpreted to be the result of edema. The Kupffer cells were not strikingly enlarged nor did they contain unusually large quantities of pigment. In one instance, from sub-group 112, there was a heavy periportal infiltration of plasma cells.

*Kidneys (Table XIII).* Hemorrhages were observed beneath the mucous membrane of the renal pelvis in most instances; otherwise there was nothing remarkable grossly.

TABLE XIII  
*Hiroshima, Group I: Kidneys*

Observations	Sub-group		
	111	112	122
Gross specimens available	3	8	0
Histologic specimens available	2	7	0
Hemorrhages of pelvis	2	6	
Cloudy swelling	2	7	
Small and large mononuclear cells in corticomedullary sinusoids	1	2	
Heavy plasma cell infiltration		1	

Except for cloudy swelling of the epithelial cells of the convoluted tubules in many cases, there were no significant microscopic changes. The blood vessels did not contain fibrinoid or hyaline material. In the sinusoids of the corticomedullary junction in several sections there were remarkable collections of small and large mononuclear elements (Fig. 96). Some were small and had almost spherical, deeply basophilic nuclei and scanty basophilic cytoplasm. These resembled small lymphocytes. Mingled with them were much larger elements whose cytoplasm was neutrophilic and at times vacuolated. The nucleus was small, ovoid or bean-shaped, and its chromatin was delicately reticular. These cells had the structure of macrophages. Other cells possessed deeply basophilic cytoplasm. Their nuclei exhibited a coarse network of chromatin, with prominent nucleoli. Such cells resembled "blasts." Occasionally some of the blast-like cells were attached to the walls of these sinusoids, and transitional forms were seen which were flattened and gave the impression of being endothelial cells in process of becoming detached and rounded. Occasionally a cell was found in mitosis. There were no cells that had the appearance of mature normoblasts nor was there granulation of the cytoplasm to indicate that they were myelocytes. We have noted similar cells in similar positions in various other conditions. They have been noted also in scrub typhus.<sup>2</sup> In one patient who had severe burns there was a very heavy plasma cell infiltration about the glomeruli. These

cells occurred also in large numbers elsewhere in the tissues of this patient, as in the spleen and heart.

*Ureters and Bladder.* No changes were observed in the ureters and bladder.

*Testes.* Grossly, in the 4 instances in which material was available, the tubules of the testes strung out easily and no changes in size or consistency were noted.

Microscopically, in 2 of the 3 specimens available, there were striking changes, even after 4 days. The germinal epithelium had largely sloughed from the basement membrane. Sertoli cells had become prominent and in some places formed a continuous sheet. The spermatogonia were largely in the lumina of the tubules. Many were still found in mitosis, but spermatids and spermatozoa already seemed diminished in numbers (Fig. 98). Many spermatogonia and their derivatives possessed pyknotic nuclei. The Leydig cells had their usual appearance. The rete testis contained not only spermatozoa but remnants of their precursors that had sloughed away in various stages of necrosis. Such changes were noted in 2 of the 3 specimens available for microscopic examination, one each from sub-groups 111 and 112. In another of sub-group 112 the tissue was poorly preserved and no definite microscopic changes were discovered.

*Ovaries.* In the ovaries of one patient, 13 years of age, who probably had not reached the menarche, there were numerous primordial follicles and small follicular cysts, but no developing follicles or corpora albicantia. No other specimens were available for histologic study.

*Brain (Table XIV).* Striking congestion of the vessels was usually described upon gross examination of the brain. In one instance, there were superficial contusions and hemorrhages in the occipital and temporal lobes, probably of traumatic origin. In another, capillary hemor-

TABLE XIV  
*Hiroshima, Group I: Brain*

Observations	Sub-group		
	111	112	122
Gross specimens available	2	7	0
Histologic specimens available	2	5	0
Congestion	2	4	
Edema of pia		2	
Multiple contusions with hemorrhages	1		
Capillary hemorrhages		1	

rhages were found. Histologically, neither cellular exudate nor gliosis was in evidence and the ganglion cells, including the Purkinje cells, were well preserved.

*Adrenals (Table XV).* In the adrenal glands of even the earliest cases there often was evidence of a decrease in cortical lipid. Grossly, this was shown by the pale yellow-gray, rather than orange-yellow, color of the cortex and by its remarkable translucency.

TABLE XV  
*Hiroshima, Group I: Adrenals*

Observations	Sub-group		
	III	II2	I22
Histologic specimens available	3	6	I
Atrophy of cortex*	I	3	I
Fibrinous material in peri-adrenal fat	I		
Heavy plasma cell infiltration		I	
Hyaline change of capsular arterioles		I	

\* Usually with special involvement of the zona glomerulosa.

Microscopically, as early as the fourth day, there was evident a striking atrophy of the cells in the outer cortical zone, and edematous collagenous material extended inward from the capsule (Fig. 116). This change resembled that of the pericentral tissues of the liver (compare with Fig. 93). The other cortical cells also showed relatively little vacuolation and had finely granular cytoplasm. There was an abundance of finely granular brown pigment in the reticularis. The medullary substance was preserved in its typical histologic appearance. In another case, which was not otherwise unusual, fibrinous material had appeared in the septa of areolar tissue that traversed the periadrenal fat. Hyaline changes of the arterioles of the capsule were found in another. A third adrenal gland, from a severely burned patient, showed a striking infiltration of plasma cells.

*Thyroid Gland.* No changes were detected grossly or microscopically in the thyroid gland.

*Parathyroid Gland.* The one parathyroid gland available, from a patient in sub-group 112, was found to consist almost entirely of chief cells of typical appearance.

*Pituitary Body.* Three sections were available from the pituitary body, one from a patient in sub-group 111 who died on the fourth day. Despite poor fixation, it was evident that acidophilic cells predominated, which is to be expected in a 24-year-old man. The other two, one from a female and the other from a male patient, likewise showed a predominance of acidophilic cells.

*Thymus.* Interpretation of changes in the thymus always is difficult. The structure of the organ in a 13-year-old boy who died on the third day resembled that of the lymphoid tissue. The connective tissue was

edematous. The lobules of the parenchyma consisted of stellate reticular elements supporting smaller cells with ovoid nuclei which likewise had stellate processes. Small round cells indistinguishable from lymphocytes were rare, although they should be present in large numbers at this age. Occasional mononuclear cells with eosinophilic granules in the cytoplasm were found. Hassall's corpuscles were numerous but small. They were anuclear at their centers in most instances, and some had become calcified.

In other specimens from this group, according to the gross descriptions, almost total atrophy of the organ had occurred, but no sections were available for study.

*Neck Organs.* Remarkable changes had occurred in the epithelium of the pharynx, tonsils, tongue, and esophagus of K-98, the single case available for detailed study.\* Unfortunately, the distance from the bomb and other exposure factors are unknown. It was stated that this man had sustained trauma and burns. It may be presumed from the appearance of the lymph nodes and the sternal bone marrow (Figs. 44 and 58) that a massive dose of ionizing radiations was received.

In the *pharynx* of this patient the epithelial cells had become remarkably swollen, vacuolated, and fragmented as had their nuclei, and much of the epithelium had desquamated. The connective tissues were tremendously edematous and there was a striking lymphectasia. Scattered plasma cells and large mononuclear cells occurred in the areolar tissue. The lymphoid tissue had become remarkably atrophic here as elsewhere (Figs. 119 and 120).

In the *tongue* the epithelium consisted of enormous, bizarre epithelial cells whose cell boundaries were now difficult to distinguish (Fig. 121). Many of the nuclei had become multi-lobed and in each lobe there was a prominent nucleolus. Multiple minute nuclei, resembling the "micro-nuclei" observed as a colchicine effect,<sup>14,80</sup> were present in some large cells. Some cells had become separated from their neighbors and appeared as refractile, homogeneous, acidophilic masses. Thick layers of parakeratotic material were present on the surface. In the connective tissue underlying the epithelium there were dilated lymphatics and small, closely crowded, spindle-shaped fibroblasts.

The epithelial cells lining the crypts of the *tonsils* exhibited changes similar to those observed in the pharynx. Here also, the nuclei were vesicular, with prominent knots of chromatin. There was a remarkable alteration of the lymphoid tissue, which was represented largely by

\* Sections of the tonsils from another patient exhibited only atrophy of the lymphoid tissue but no notable epithelial changes.

compacted spindle-shaped elements of the reticulum among which were suspended only a few mature lymphocytes. The substance of the tonsil had not become necrotic.

There were notable changes in the *esophagus*. In many places the squamous epithelium of the surface had been replaced by purple-staining, dull, necrotic material. Only the swollen remnants of a few squamous cells, as bizarre in their cytoplasmic and nuclear components as those in the tongue, could still be distinguished. In the immediately underlying tissue there was a striking degree of edema, which extended deeply among the bundles of striated muscle, and there also was dilatation of vessels. There was no leukocytic infiltration.

With certain qualities of radiation these mucous membranes are more prone to show demonstrable changes in the epithelium than the skin (Coutard<sup>18</sup>). Unfortunately, sections of skin from this patient (K-98) were not available.

The changes in the tissues of K-98 undoubtedly represent radiation effects. They are analogous to those in the intestine as shown in Figures 80 and 82. In the present instance, however, there were no atypical cells or mitotic figures in the stomach, which represents the only portion of the gastro-intestinal tract available in histologic sections.

*Skin.* Unfortunately, skin unaltered by thermal burns is not available from any patient dying during the first 2 weeks.

#### *Group II: Patients Dying During the Third, Fourth, Fifth, and Sixth Weeks*

In group II, changes characteristic of radiation, such as epilation, testicular atrophy, and, particularly, damage to the hemopoietic tissues were at their peak. The most striking new developments were those associated with the infections that accompanied the aplastic anemia. All surfaces to which bacteria had access became the seat of foci of necrosis accompanied by hemorrhage. Ultimately there was evidence in many cases of generalization of the infection. If there was any leukocytic response, it was of plasma cell or mononuclear type. Extravasations of blood also occurred apart from any obvious local foci of infection in such organs as the kidney. The factors—heparin, platelets, bacteria, and vitamins—possibly concerned in the pathogenesis of the purpura are discussed elsewhere.<sup>47</sup> It is of note that the blood at necropsy often was observed to be in an unclotted state.

The salient clinical data relevant to the Hiroshima patients who ultimately were necropsied are recorded in Table XVI, in which all groups are compared.

TABLE XVI  
Recorded Clinical Data on Hiroshima Patients, Examined by Necropsy

Observations		Group I			Group II			Group III			
		Sub-group			Sub-group			311	Sub-group		
		111	112	122	211	212	222		312	321	322
Total number		3	8	1	58	5	2	9	1	4	3
Male		3	7	0†	46	5	1	5	1	3	2
Female		0	1	1	12	0	1	4	0	1	1
Well nourished					42	4	1	4	0	0	0
Poorly nourished					16	1	1	5	1	4	3
Burns		3*	8†	1†	10*	5†	2†		1†	1*	3†
Mechanical injury		3	1		33			5			
Nausea and vomiting on day of bombing		2	4		11						
Epilation		0	0	0	50	1		6		1	1
Purpura		0	0		38	1		2			
Ulcers or abscesses of skin		0	0		3				1		
Gingivitis (all)		0	0		37			2			
Gingivitis (necrotic)		0	0		3						
Pharyngitis or tonsillitis (all)					32			1			
Pharyngitis or tonsillitis (necrotic)		0	0		5						
Diarrhea (all)		0	5	1	24	3		4		3	1
Diarrhea (hemorrhagic)					11	1		2		2	
Epistaxis					6	3		1			
Rectal hemorrhage					1						
Vaginal hemorrhage					2						
Hemoptysis					4			1			
Fever (maximum)	To 39°C.		1								
	To 40°C.		1	1	21	3	1				
	Over 40°C.		2		6	1		2			
Fever present, temperature unstated		2	2		17						1
Lowest white blood cell count recorded	0-500				20			1			
	501-1000				6	2		1			
	1001-1500				2	2					
	1501-2000							1			
	2001-2500				1						
	2501-3000										
Lowest red blood cell count recorded	Over 3000	1	2	1			1	3		1	
	Less than 1.6				2			3			
	1.6-2.0										
	2.0-2.5				9			1			
	2.6-3.0				5					1	
	3.1-3.5				6						
	Over 3.5				5	3	1	1			

\* Slight.

† Severe.

‡ 0 indicates that the sign or symptom was not present. A blank space indicates that no observation was recorded in the clinical record. The incidence of any finding as stated in the table is minimal, since the basic information may be incomplete in some instances.

*Heart (Table XVII).* Epicardial hemorrhages were usually, and endocardial extravasations sometimes, present (Fig. 10) and occasionally there also were hemorrhages in the neighborhood of the conduction bundle (Fig. 16). Fluid blood, which did not clot upon standing, frequently was observed in the chambers of the heart and great vessels. Perivascular hemorrhages in the myocardium occasionally were present

TABLE XVII  
Hiroshima, Group II: Heart

Observations	Sub-group		
	211	212	222
Gross specimens available	55	5	2
Histologic specimens available	49	5	2
Epicardial hemorrhages*	33	3	
Endocardial hemorrhages*	6		
Fluid blood in chambers*	8	2	
Perivascular edema	4		
Perivascular hemorrhages of myocardium	7		
Thrombus in venule			1
Focal necrosis of myocardium	1		1
Plasma or mononuclear cell infiltration of myocardium	1		1
Plasma or mononuclear cell infiltration of endocardium	7	1	
Additional diagnoses			
Chronic verrucous endocarditis	2		

\* Gross diagnosis.

and there sometimes was edema of the perivascular tissue, as observed in the specimens from patients dying before the end of the second week. In 5 instances, there was seen immediately beneath the endothelium of the chambers an exudate of plasma cells sometimes mingled with small and large mononuclear cells. These also were present in the myocardium (Fig. 21).

In 2 instances there was evidence of previous rheumatic disease, in the form of chronic verrucous endocarditis.

*Lungs (Table XVIII).* The characteristic gross lesion of the lungs in group II was that of focal necrosis centered upon minute bronchioles whose lining membranes had become necrotic. Surrounding these foci of necrosis were brilliant red zones of hemorrhage which had become confluent in some instances. The intervening parenchyma was translucent and pale, and exuded a large quantity of fluid which was only slightly cloudy. In some instances hemorrhage was predominant about extremely minute foci of necrosis (Fig. 19), but in others dull yellow or gray-green, opaque, rounded masses of necrotic tissue were surrounded by relatively narrow zones of extravasated blood (Fig. 17). Tuberculous lesions also may be surrounded by massive hemorrhages at this stage, so that vigilance was necessary in gross interpretation to avoid confusion with ordinary non-tuberculous necrosis (Fig. 18).

The pleura usually was the seat of extravasation of blood and was rendered thick and translucent by edema, which produced similar changes in the septa.

Histologically, these lungs had a characteristic structure to which the term "neutropenic pneumonia" has been applied. There was a striking degree of edema of the pleura and peribronchial and perivascular



TABLE XVIII  
*Hiroshima, Group II: Lungs*

Observations	Sub-group		
	211	212	222
Histologic specimens available	57	5	2
Focal atelectasis and emphysema	1	1	
Edema of interstitial tissue (no other lesions)	1		
Edema of alveoli	1		
Focal parenchymal hemorrhages and edema	11*		1
Focal "neutropenic pneumonia"	31	1	
Focal necrotizing pneumonia with polymorphonuclear cells	3	2	
Focal tuberculous pneumonia	2		
Fibrinous pleurisy	4	1	
Fibrinopurulent pleurisy	1		
Hemorrhage in pleural cavity	1		
Serous pleural effusion	1		
Additional diagnoses			
Fibrous pleural adhesions	13	1	
Healed tuberculous foci	6		
Organizing tuberculous pleurisy	1		
Tuberculosis of hilar lymph nodes	2	2	
Ascaris in pleura	1		

\* Six of these patients had severe ulcerative enteritis and five others had necrotizing tonsillitis to account for death.

tissues. The parenchyma showed large foci of necrosis which were centered upon the bronchioles, whose lumina were filled with finely granular, pink-staining material containing large clumps of bacteria (Fig. 22). The lining membranes of these bronchioles had become completely necrotic save that portions of the basement membrane persisted. In the vicinity of these bronchioles the shadowy remnants of the walls of the alveoli could still be discerned, but in some places these had become invisible. The alveoli were filled with fibrin, ensnaring large numbers of erythrocytes. No polymorphonuclear leukocytes were found, although large mononuclear elements with phagocytized, finely granular, brown pigment were scattered throughout the tissue.

In some instances the alveoli contained much extravasated blood (Fig. 23), but in others there were relatively few erythrocytes, but relatively much amorphous acidophilic substance, and fibrin was abundant (Fig. 24).

The lungs of 5 patients who presumably had been exposed to radiation exhibited a focal necrotizing pneumonitis in which polymorphonuclear cells were abundant. In these patients the bone marrow was found not to be hypoplastic.

Two patients had a focal caseous tuberculous pneumonitis. It is interesting that the exudate in these lungs contained numerous large mononuclear cells, although polymorphonuclear leukocytes were absent.

The caseous foci were surrounded by large extravasations of blood. Tuberculous foci in other irradiated patients of this group remained quiescent (Table XVIII).

*Spleen (Table XIX).* Usually the malpighian corpuscles remained in a state of atrophy which sometimes was extreme and which might possess all of the features manifest in group I, as late as the 18th day after irradiation (Fig. 42). Atypical large mononuclear cells, such as have been described, frequently were found in large numbers (Fig. 43). In some spleens there were evidences of regeneration (Figs. 41, 45, 46, and 47). The regeneration was of a remarkable type. It appeared at first to be associated with condensation of syncytial spindle-shaped reticulum cells upon the almost naked central arterioles of the malpighian corpuscles. Occasional mitotic figures were found among these reticulum cells. Small lymphocytes were seen in close association with them and indeed seemed to be derived from them. The lymphocytes appeared first between the syncytium and the central arteriole, but also in a halo

TABLE XIX  
*Hiroshima, Group II: Spleen*

Observations	Sub-group		
	211	212	222
Histologic specimens available	51	5	2
Perisplenic adhesions	4		
Decrease of lymphocytes	48	3	1
Absence of germinal centers	49		
Atypical mononuclear cells	13	2	2
Heavy plasma cell infiltration	7		
Regeneration with perifollicular condensation of reticulum	8		
Infarct		1	

about the former. Their number increased centrally and it appeared for a time as if the "germinal center" was at the periphery of the follicle. In larger follicles, presumably later in regeneration, minute germinal centers reappeared in their central positions within the follicle.

A morphologically similar "hematopoietic perifollicular envelope" recently has been described in the spleen of the rat by Krumbhaar.<sup>48</sup> In the human spleen only lymphocytes appear to be derived from such cells, while in the rat they may also be precursors of normoblasts and granulocytes.

*Lymph Nodes (Table XX).* Between the end of the second and sixth weeks, there was usually a continued absence not only of the germinal centers of lymph nodes, but also of typical small lymphocytes. The tendency toward shrinkage on this account was counteracted by pro-

TABLE XX  
Hiroshima, Group II: Lymph Nodes

Observations	Sub-group		
	211	212	222
Microscopic specimens available	39	2	1
Marked enlargement of nodes	2*	2	1
Large nodes composed of typical lymphocytes, but without germinal centers	1		
Decrease of lymphocytes	36		
Absence of germinal centers	36		
Germinal centers present	2		
Atypical mononuclear cells	25	1	1
Bacterial masses in node, with necrosis	2		
Additional observations			
Tuberculosis	2		

\* K-22 and K-23.

liferation of atypical cells. In 5 instances this proliferative process was so extensive as to produce nodes of several times the usual size. Thus the bare reticular skeleton found in some of the earlier cases only rarely persisted as long as 2 weeks (Fig. 33). On the contrary, the node supported great numbers of atypical large cells, some resembling lymphoblasts (Figs. 49 and 50), some with plasmacytoid characteristics, and others representing very bizarre polymorphous derivatives of the reticulum (Figs. 51 and 52), occasionally with the structure of Reed-Sternberg cells (Fig. 53). Forms intermediate between these and ordinary reticulum cells were common. The inception of this proliferative activity was noted during the first 2 weeks, but now had reached its acme. In Giemsa-stained preparations numerous eosinophils and many mast cells of variable size and shape were present (Fig. 33). Some of the latter were remarkably elongated.

*Bone Marrow (Table XXI).* The tissue available is unique, since relatively little has been published concerning the effects upon the bone marrow in man of single massive doses of ionizing radiations to the whole body. Despite the devastation of the hemopoietic tissues that may occur soon after irradiation, the marrow in later weeks displayed a remarkable regenerative capacity. At first, as in the spleen and lymph nodes, the proliferative activity resulted in the production of atypical cells only. Most marrows in group II patients were of this type. In some marrows there was evidence of direct transformation of reticulum cells into myelocytes (basophilic blast cells being minimal in number), as well as into plasma cells or lymphocytoid elements as described by Rohr.<sup>76</sup> Some marrows, however, especially in patients dying in the fifth and sixth weeks, showed moderate or even extreme myeloid hyperplasia,

TABLE XXI  
Hiroshima, Group II: Bone Marrow

Observations	Sub-group		
	211	212	222
Long bones			
Histologic specimens available	27	2	0
Type A: hypoplasia	18	1	
Type B: marked focal reticulum hyperplasia	3		
Type C: focal myeloid regeneration	5		
Type D: marked myeloid hyperplasia	1	1	
Flat bones			
Histologic specimens available	22	3	1
Type A: hypoplasia	6	2	1
Type B: marked focal reticulum hyperplasia	5		
Type C: focal myeloid regeneration	10		
Type D: marked myeloid hyperplasia	1	1	
No marrow			
Tissue response "aplastic"	5		
Polymorphonuclear cells in tissue	2		1

but even in such instances there might be evidence of a "maturation defect," with persistence of peripheral counts below 2000. The marrow of the long bones took part in the regenerative process.

The marrows of group II may be classified according to the degree and type of regeneration as follows:

Type A. Marrows showing marked hypoplasia

Type B. Marrows showing marked focal reticulum hyperplasia

Type C. Marrows showing focal myeloid regeneration

Type D. Marrows showing myeloid hyperplasia

The available material consisted of sections of tissue from either a long or flat bone, in many instances supplemented by smears made from the bones post mortem and stained by the Giemsa or Wright-Giemsa methods. If a flat bone was available, the case was classified according to the histologic features of that bone. If only material from a long bone was at hand, the case was classified according to its histologic appearance but additional information, derived from examination of supplementary smears from the rib, sternum, or vertebra, was applied when it was available.

In 8 of the patients of group II, neither marrow tissue nor smears were available. In those cases, foci of necrosis in the lung, intestine, or skin were examined to determine whether polymorphonuclear leukocytes were present. It will be recalled that even hyperplastic marrow may deliver remarkably few polymorphonuclear cells to the tissue.

The numbers in the various categories are shown in Table XXI. They will now be discussed in turn.

Type A. Some marrows still retained the almost totally aplastic character exhibited by many in the first stage, with only minimal evidence of the proliferation of reticulum and of the formation of varying numbers of plasmacytoid and lymphoid cells. This change was observed even in the ribs, sternum, and vertebrae (Figs. 55 and 56). Grossly, such a hypoplastic marrow had a gelatinous, extremely translucent, pale red appearance, and the bone yielded only a slightly cloudy, almost serous fluid upon scraping or squeezing, in contrast to the abundant, pasty, gray-red material that usually is obtained.

In some instances, with further proliferation, the reticulum and its derivatives began to form thin septa of proliferating tissues among the fat cells. Some of the reticulum cells tended to become round and to be divorced from their fellows while the nuclear membrane became thicker and knots of chromatin became more prominent in some of the larger cells. The chromatin tended to clump at the periphery and all transitional stages could be demonstrated between such elements and the typical ovoid plasma cells on the one hand and the reticulum cells on the other (Fig. 61). Rarely, and to a slight extent, there was differentiation also into myeloid elements. In most of the aplastic cases the megakaryocytes had almost completely disappeared or they might be represented by small stellate elements with vesicular nuclei and faintly acidophilic or neutrophilic cytoplasm about which fibrin tended to be deposited (Fig. 73). Islets of erythropoiesis often persisted despite extreme atrophy of the remainder of the tissue (Fig. 60). In some instances, even they had all but disappeared (Fig. 56). Clinically, these patients all had a profound leukopenia and a moderate or severe anemia.

Type B. In another type, which differs only quantitatively from that just described, there was a striking focal hyperplasia of the reticulum, and the derivative plasma cells and lymphocytoid elements might be prominent in the cellular population. There also was evidence in some instances of differentiation into myeloid cells. In part the marrow was hypoplastic, but elsewhere, particularly near the trabeculae of bone, there had occurred a tremendous proliferation of large stellate cells, whose nuclei varied somewhat in structure (Figs. 62 to 64). Many were large and ovoid, with thin nuclear membranes and delicately reticular chromatin. Others had somewhat thicker nuclear membranes and more prominent knots of chromatin. Despite the stellate shape of the associated cytoplasm, these resembled the nuclei of plasma cells, and there also were large rounded cells whose general structure was plasmacytoid and

some that resembled lymphocytes more than plasma cells. A few cells with scanty basophilic cytoplasm, thick nuclear membranes, and prominent nucleoli, which were thought to be blast cells, also were present. In Giemsa-stained smear preparations some cells were found to contain the fine azurophilic granules of undifferentiated myelocytes. Metamyelocytes were extremely rare. Evidence for the origin of the myelocytes from the stellate cells of the reticulum was the presence of cells transitional between the two, which contained a few of the azurophilic granules (Figs. 36 and 65). There were a few mast cells with numerous prominent basophilic granules closely resembling those of the lymph nodes. A few small islands of erythropoietic tissue also were found. There were also some large and small cells with complexly folded nuclei (Fig. 64). Their cytoplasm was still faintly basophilic and their structure suggested that of megakaryocytes, but some of the smaller cells were intermediate in appearance between megakaryocytes and reticulum cells.

Bacteria were found in 2 cases: streptococci in one (Fig. 66) and thin elongated bacilli in the other (Figs. 67 and 68). Both marrows were well fixed immediately post mortem. In the latter there was necrosis of the tissue, but there was no special leukocytic infiltration about the organisms in either instance. These cases demonstrate the occurrence of bacteremia, which probably was common terminally.

The tremendous focal hyperplasia of reticulum cells may suggest the appearance of "reticulo-endotheliosis" or leukemia. In that instance, involvement of the lymph nodes or other tissues rich in reticulum cells would be expected. In all cases in which "type B" marrow was encountered, however, the Kupffer cells of the liver showed little more than a moderate degree of erythrophagia and hemosiderosis, and there was no unusual proliferation of these cells. In one case with type B marrow, K-22, some of the nodes were markedly enlarged and contained numerous atypical mononuclear and reticular cells. These, however, did not differ from the atypical cells of the spleens and nodes of many other patients in whom the bone marrow was notably hypoplastic. Unfortunately, no leukocyte counts are available for K-22, but there appeared to be some differentiation of the reticulum into myeloid tissue, despite the rarity of the blast cells. It seems preferable to regard this proliferated reticulum merely as hyperplastic tissue, little differentiated, but without obvious neoplastic properties.

A single case of leukemia of monocytic type occurred among the approximately 14,000 patients studied clinically in the two cities. This case is presented in group III.

Type C. In a third type, myeloid tissue definitely predominated in

small foci where regeneration was in progress. In these foci there might still be evidence of proliferated reticulum, and excessive numbers of plasma cells and lymphocytes still were present. Myeloid hyperplasia in this instance was focal, and there was maturation at least into metamyelocytes. Megakaryocytes appeared in increasing numbers. There was no sharp separation between the marrows of this type and those of type A or B.

Occasionally, in the Nagasaki series, eosinophils, mature and immature, were prominent in such marrows, particularly in group III (patients dying after the sixth week).

Type D. In the fourth type, seen rarely in this group, hyperplasia was extreme and the fat cells were hardly visible amid the masses of myelocytes, and in some instances metamyelocytes. The cytologic characteristics of this marrow did not differ essentially from that of type C, except for predominance of the more mature cells.

The last two types became predominant after the sixth week, and will be described and illustrated in group III.

Comparison of Tissue from Various Bones. Cell counts of bone marrow smears made at autopsy are summarized in Table XXII and in Text-Figure 1. They are grouped according to classification of histologic specimens. Thus, by reading the chart horizontally, the cytologic features of the various bones in a particular type of case can readily be compared. The columns read vertically show variations in the counts for any particular bone from case to case.

A common feature was the great increase in the reticular and lymphoid tissue, largely at the expense of the myeloid tissue. The erythroid tissue also had suffered decrease but the reduction was less in comparison with normal values.

The femur took part in the regenerative process along with the flat bones, and in some instances hyperplasia, reticular or otherwise, in the femur was extreme. Usually the smears from the flat bones conformed to the histologic structure of the long bones; in some instances there was relatively more myeloid tissue in the former. Even from long bones, however, smears showed the same contrast with fixed tissue, probably because the free, more differentiated cells are yielded more readily to the glass than are the reticular elements.

Of all flat bones, the sternum showed the earliest regeneration and the greatest cellularity, but occasionally even the femur exceeded the sternum in these respects.

TABLE XXII  
*Hiroshima, Group II\*: Comparison of Fixed Tissues and  
 Post-Mortem Smears of Bone Marrow*

Source and differential count† of smear					Histology of fixed tissue
Cell type	Sternum	Rib	Vertebra	Femur	
M	12.0	6.6			Femur, type A marrow
L	31.5	43.5			
R	53.1	45.8			
E	3.4	4.1			
	(Average of K-26, 27, 31)	(Average of K-21, 24, 25)			
M	6.0			18.0	Femur, type A marrow (K-30)
L	29.0			30.0	
R	60.0			48.4	
E	5.0			3.6	
M	21.6		7.0	7.0	Sternum, type A marrow (K-42)
L	33.6		49.0	49.0	
R	28.8		34.0	34.0	
E	16.0		10.0	10.0	
M		21.1		32.2	Vertebra, type A marrow (K-37)
L		44.0		38.2	
R		32.8		28.6	
E		2.1		1.0	
M		24.8			Femur, type B marrow
L		30.5			
R		31.7			
E		13.0			
		(Average of K-29, 44)			
M			51.5		Vertebra, type B marrow (K-22)
L			13.5		
R			27.0		
E			8.0		
M	5.0			19.0	Sternum, type B marrow (K-43)
L	35.0			35.0	
R	55.0			42.0	
E	4.0			4.0	
M	29.3				Femur, type C marrow (K-32)
L	21.6				
R	43.1				
E	5.0				

\* This series of specimens is derived from patients dying during the third and fourth weeks.

† Counted by Dr. G. V. LeRoy.

Key: M = myeloid cells; L = lymphoid cells; R = reticular cells (reticulum and plasma cells, and intermediate forms); E = erythroid cells. Types A, B, and C marrows are defined in text.

In drawing conclusions from this material, both from histologic and smear preparations, it must be remembered that one is dealing with very small and possibly unrepresentative portions of the entire vast body of the marrow.

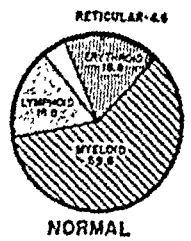


# HIROSHIMA

## COMPARISON OF FIXED TISSUES AND SMEARS OF BONE MARROW

### GROUP II

(Patients dying during 3rd. & 4th. week)



SOURCE, & DIFFERENTIAL COUNT* OF SMEAR				HISTOLOGY of FIXED TISSUE
Sternum	Rib	Vertebro	Femur	
<p>AVERAGE OF KEY Nos. 26, 27, 31</p>	<p>AVERAGE OF KEY Nos. 21, 24, 25</p>			Femur Type A
			<p>(KEY No. 30)</p>	Femur Type A
				Sternum Type A (KEY No. 42)
			<p>(KEY No. 37)</p>	Vertebro Type A
	<p>AVERAGE OF KEY Nos. 28 and 44</p>			Femur Type B
				Vertebro Type B (KEY No. 22)
			<p>(KEY No. 45)</p>	Sternum Type B
				Femur Type C (KEY No. 32)

\* COUNTED BY DR. G. V. LE ROY

Relation of Leukocyte Counts to the Histologic Structure of the Marrow. Reference to Table XVI will show that in all but one of the 34 patients in group II for whom leukocyte counts have been recorded, the count had been 2500 or less at some time before death and in most of these patients it was below 1000. This leukopenia had no relation to the type of marrow ultimately found at necropsy.

Maturation Defects. In certain instances, there was striking evidence of maturation defect, for, despite diffuse myeloid hyperplasia, peripheral counts sometimes remained remarkably low. The history of K-108 is detailed as an example.

The patient was a 29-year-old man, who was at a distance of 700 yds. from the hypocenter. He was outdoors a few paces from a concrete building. He was struck by fragments of a falling roof which inflicted slight injuries to the head and neck. There was nausea on August 6 (the day of the bombing), and on the same day he vomited between 20 and 30 times. Malaise began on August 6 and lasted until the 10th, accompanied by anorexia. He again experienced malaise beginning on August 21 until the time of death. Anorexia appeared 4 days after the second onset of malaise. There was epilation, and gingivitis on August 21, which persisted. The gingivae began to bleed on August 30. On the 25th purpuric manifestations began and there was evidence of tonsillitis, both symptoms lasting until death on September 1. There was high fever between August 24 and the time of death, and there was cough and sputum beginning on the 25th, with hemoptysis on August 30.

	Red blood cells	White blood cells
August 24	3.95 millions	370
August 26	5.64 millions	450
August 29	4.19 millions	200
August 30		220

The urine examined on August 29 was positive for albumin and negative for sugar. No statement was made concerning sediment.

The marrow of the patient, in sections derived apparently from the cavity of a long bone, was of type D, showing vascular adipose tissue crowded by very large numbers of young myelocytes. Mature polymorphonuclear leukocytes and even band cells were rare. There was an occasional megakaryocyte. Occasional cells were found in mitosis. A few small nests of shrunken nuclei, thought to be those of normoblasts, also were found. Other significant lesions at necropsy were petechiae of the skin, epilation of scalp, focal necrosis of pharynx, tongue, tonsils, and larynx, necrotizing gingivitis, an abscess in the region of the right mandibular joint, necrotizing and hemorrhagic neutropenic pneumonia, and minute hemorrhages of the gastro-intestinal tract, trachea, and renal pelvis.

Rising Counts. Occasionally, however, patients who had had a profound leukopenia manifested a rise in count to approximately 5000, before death. This is demonstrated in K-86 (sub-group 211).

At the time of the bombing, the patient, an 18-year-old girl, was at home in bed at Kawaramachi, approximately 800 yds. from the hypocenter. She was injured by fragments of glass which entered both upper extremities, the left submaxillary region, the back, and the left knee joint. She lost consciousness for a time. The wounds progressed favorably, but on August 20 she noted epilation and suffered diarrhea. Suddenly in the night of August 30 there was high fever, accompanied by severe sore

throat. She was admitted to Iwakuni Naval Hospital on August 31, 1945. On that date the white blood cells numbered 960 per cmm.; on the next day, 620; then they fell daily to a minimum of 100 on September 5, 1945. After that the count gradually increased to 480, 1680, 3200, and 7980. Her general condition, however, worsened and she died on September 9.

At necropsy the bone marrow showed a moderate focal hyperplasia with myeloid differentiation (type C). The other findings included epilation of scalp, scattered petechiae of the viscera, atrophy of the lymphoid tissue, and a necrotizing focal pneumonia.

A rising leukocyte count is much more frequently recorded in patients dying later (group III). Obviously, moreover, many patients, in whom the marrow, after a period of depression, became capable of producing a leukocytosis, recovered and escaped inclusion in the autopsy series.

*Gastro-intestinal Tract (Table XXIII).* Hemorrhages and ulceration occurred very frequently in the gastro-intestinal tract in patients dying

TABLE XXIII  
*Hiroshima, Group II: Gastro-intestinal Tract*

Observations	Sub-group		
	211	212	222
<b>Stomach</b>			
Gross specimens available	58	5	2
Petechiae	36	2	
Diffuse necrosis and hemorrhage	1		
Ulcers and hemorrhages	4		
Plasma cell infiltration	1		
<b>Small intestine</b>			
Gross specimens available	58	5	2
Petechiae	19		
Ulcers and hemorrhages	7		
<b>Large intestine</b>			
Gross specimens available	58	5	2
Petechiae	14		
Ulcers and hemorrhages	26		1
<b>Additional diagnoses</b>			
Ascariasis	16	1	
Chronic ulcer of pylorus	1		
Perforation (agonal) of cardia	1		
Scars of ileum	1		
Absence of appendix	1		

between the third and seventh weeks. The tissue response usually was neutropenic. In their pathogenesis these lesions probably represented the results of infection associated with the aplastic anemia, perhaps initiated in some cases by the direct action of the ionizing radiations upon the epithelium. Beyond the second week, however, the atypical epithelial cells found earlier were no longer in evidence.

Petechiae almost always were present in the stomach. They were most numerous in the vicinity of the magenstrasse, but occurred also elsewhere in the mucous membrane. Ulcerative lesions occurred occa-

sionally in the stomach, and the tissues at the base and margins of the ulcers were suffused with blood. At times necrosis and hemorrhage were diffuse, with great swelling and induration of the mucous membrane (Fig. 84). The surface assumed a dull gray-green, lusterless appearance which bordered sharply upon typical pale-pink mucous membrane. The necrotic material was underlain by a strikingly edematous and hemorrhagic mucosa. Histologically, almost no polymorphonuclear cells were found (Fig. 89) but there were numerous plasma cells in the lamina propria. Among these there were also spindle-shaped elements with nuclei resembling those of the plasma cells but with elongated bodies of cytoplasm like those of fibroblasts. Near the mouths of the glands many of the epithelial cells were found in mitosis. Most of these were in metaphase and did not appear to be atypical. At the very surface in some places there was necrosis and hemorrhage which extended halfway to the muscularis mucosae. Purple-staining bacterial masses existed in the superficial portions of the necrotic material, but no leukocytic barrier delimited the latter. The submucosa was greatly thickened by edema, and there were present large stellate elements, fibroblasts, macrophages, and mast cells. The bundles of the muscularis also were widely separated by edematous connective tissue. Irregular hemorrhages were present within the submucosa also and there were scattered lymphocytes, plasma cells, and rare eosinophils in the superficial portions of this tissue near the muscularis mucosae.

In the small intestine also there were foci of necrosis, usually discrete. They were most numerous in the region of the ileocecal valve where there was almost always involvement (Figs. 85 to 87).

In the large intestine, which was most frequently involved, the necrosis might be diffuse (Fig. 88) but the lesions usually were focal. Often there was a polypoid projection of edematous hemorrhagic tissue into the lumen of the bowel rather than an ulcer crater. The projecting mass was covered by an opaque, ashen, yellow or gray-green material, and at its base there was a halo of hemorrhage. Histologically, the tissue response again was usually aplastic (Fig. 91) or largely of the plasma cell type (Fig. 90).

Only in 4 instances were large numbers of polymorphonuclear leukocytes found in the exudate at the base of the intestinal ulcers. Most of these patients died toward the end of the fifth and during the sixth week, when the marrow was experiencing a measure of return of myelopoiesis.

*Liver (Table XXIV).* Contrary to the gross descriptions of the Japanese pathologists, fatty change was rarely encountered in the liver

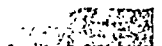


TABLE XXIV  
Hiroshima, Group II: Liver and Gallbladder

Observations	Sub-group		
	211	212	222
Histologic specimens available	55	4	1
Perihepatic fibrous adhesions	3		
Subcapsular hemorrhage	1		
Giant nuclei in pericentral hepatic cells	4		
Central congestion	24	2	
Edema of pericentral connective tissue	8	1	
Edema of periportal connective tissue	2		
Focal fatty changes: Periportal	1	2	
Midzonal	1		
Irregular	1		
Focal necrosis: Periportal	1		
Pericentral	1		
Pylephlebitis	1		
"Hyaline bodies" in cytoplasm	1		
Gallbladder			
Petechiae	1		
Additional diagnosis			
Hepatoma	1		

in this stage. It was observed microscopically in but 5 cases. Necrosis also was rare and was observed only once in pericentral and once in periportal positions. When present, the lesions were widely disseminated. The cellular exudate was scanty, had itself largely become necrotic, and consisted for the most part of plasma cells (Fig. 94). In one instance some of the large intrahepatic portal veins were involved in pylephlebitis. There was no evidence of ascariasis in that case, but there were focal necrotizing lesions of the intestine, which may well have been the source of the bacterial infection that probably was concerned in the pathogenesis of the hepatic lesions.

Slight central congestion again was frequently observed, as in group I, and there was occasionally thickening of the walls of the central veins, presumably edematous. Large nuclei were observed also in hepatic cells at the central ends of the cords.

*Pancreas.* No significant changes were observed in any pancreas seen grossly nor in the 25 that were inspected microscopically.

*Kidney (Table XXV).* Perirenal and pelvic hemorrhages were extremely common in patients dying during the second 4 weeks. Histologically, these consisted merely of erythrocytes infiltrating loose connective tissue in the capsule, and beneath the epithelium of the pelvis, without admixture of other cells. Occasionally the pelvic hemorrhages were diffuse (Fig. 97), and in some instances the ureter and bladder were involved also.

TABLE XXV  
Hiroshima, Group II: Kidney

Observations	Sub-group		
	211	212	222
Specimens available	56	5	0
Hemorrhages of pelvis	33	4	
Cloudy swelling	13	1	
Glomerular hemorrhages	6		
Hemorrhages in pyramid	1	1	
Hemorrhagic pyelonephritis	1		
Scars of kidney	17		
Abscesses (with polymorphonuclear cells)		1	
Atypical large mononuclear cells in sinusoids	1		
Additional diagnoses			
Leiomyomata of pyramids	1		
Double pelvis and ureter	1		

Glomerular hemorrhages (Fig. 97) were demonstrated in approximately 10 per cent of the kidneys of patients dying during this period. Grossly, such kidneys had the "flea-bitten" appearance found in acute glomerular nephritis. The nephrons in these cases, however, merely showed hemorrhages in the subcapsular spaces and tubules, without notable proliferative changes in the glomeruli. Often a few minute scars were found, within and about which plasma cells and occasional large mononuclear cells represented the only elements of a cellular exudate. There was often cloudy swelling of the elements of the proximal convoluted tubules which, in association with anemia, accounted for the pale appearance of most of these kidneys. In one instance there was hemorrhagic pyelonephritis associated with hemorrhage and necrosis in the prostate. A few polymorphonuclear leukocytes were found in the kidney in this instance.

The large and small mononuclear cells, so prominent in the medullary sinusoids in the kidneys of patients of group I, occurred only once in the group II series.

*Ureters and Bladder.* In 4 instances focal hemorrhages were found beneath the mucous membrane of the ureters. Such lesions also occurred in the bladder in 3 instances, in another the process was diffuse, and in a fifth there was diffuse hemorrhage associated with an acute necrotizing cystitis.

*Prostate and Seminal Vesicles.* The necrotizing hemorrhagic lesion of the prostate in the patient with necrotizing pyelonephritis has already been mentioned. A few leukocytes, chiefly unsegmented polymorphonuclear cells, were present within the bladder, but they were very rare in the kidney.

The seminal vesicles showed no changes except that in one instance the contents were noted histologically to be an acidophilic material containing almost no spermatozoa.

*Testes (Table XXVI).* In all 38 of the available specimens in this group of 52 male patients there was atrophy of the testicular substance.

TABLE XXVI  
*Hiroshima, Group II: Testes*

Observations	Sub-group		
	211	212	222
Well nourished patients			
Histologic specimens available	25	I	0
Atrophy of germinal epithelium and derivatives	25	I	
Thickening of basement membranes of tubules	4	I	
Hyaline changes of blood vessels	7		
Hyperplasia of interstitial tissue	2		
Malnourished patients			
Histologic specimens available	II	0	I
Atrophy of germinal epithelium and derivatives	II		I
Thickening of basement membrane of tubules	4		
Hyaline changes of blood vessels	I		
Hyperplasia of interstitial tissue	I		
Hemorrhage of tunica albuginea	I		

Since the effect of inanition\* is well known, an attempt was made to determine from the records whether the patients were well nourished. Inanition usually was recorded by the Japanese pathologists or could be determined, when severe, by inspecting the subepicardial, subcutaneous, or perirenal adipose tissue in histologic sections. Twelve patients were considered to be slightly or severely malnourished; the other 26 were apparently in a good state of nutrition at the time of death. The changes to be described must also be considered in the light of the fact that some degree of testicular atrophy is common in many exhausting illnesses. In these illnesses, however, it is rarely as complete as that observed here. Most of the irradiated patients had only a short bout of high fever before death, so that this could hardly be considered a factor in the atrophy of the testes, and none had generalized lesions suggestive of typhus. Allen and Spitz<sup>2</sup> described testes in their scrub typhus patients that resemble those illustrated here.

Clinical sperm counts performed some 10 weeks after irradiation on survivors who were well nourished, epilated, but apparently otherwise well, showed a close correlation between the degree of exposure as judged by distance from the bomb and the degree of hypospermia. This is con-

\* Testes of patients from the German prison camp at Dachau studied by Capt. E. B. Wert at the Army Institute of Pathology show all the changes described in this section. These patients weighed approximately 80 lbs. at the time of death.

firmatory evidence that the testicular changes represented at least in part an effect of radiation.

Only in one case was there a notable gross reduction in the size of the testes (Fig. 99). The masses of tubules appeared pale gray-tan but strung easily from the cut surface. Histologically, all of these testes showed separation of most or all of the germinal epithelium from the basement membrane, which was then lined by a continuous layer of tall Sertoli cells. Partly necrotic remnants of germinal epithelium and its derivatives were seen in the lumina of the tubules (Figs. 100 and 101). Spermatids and spermatozoa, however, sometimes remained embedded among the Sertoli cells. Occasionally there was found, near the basement membrane or in the lumen, a large ovoid cell with a densely reticular nucleus which from its structure was thought to be a relic of a germinal epithelial cell (Fig. 102).

Occasionally there occurred in the lumina of the tubules what appeared to be multinucleated giant cells. These apparently were formed by fusion of the cytoplasm of remnants of spermatids, successive stages of which are shown in Figures 103 and 104. Such a fused mass might become moulded into a rounded shape somewhat in the manner of a corpus amylaceum. The nuclei of such "giant cells" had a dull, homogeneous, non-reticular structure unlike that of viable cells. Barratt and Arnold<sup>8</sup> considered such cells in the testis of the rat after x-irradiation to be the result of atypical mitosis or amitosis of spermatocytes.

The basement membranes of the tubules within the first 5 or 6 weeks usually remained thin, but were found to be slightly thickened in a few instances (Fig. 104). In one malnourished boy of 8 years there was a remarkable thickening of the basement membrane of the tubules of the immature testes (Fig. 106). The epithelium appeared only slightly shrunken. The fate of other heavily irradiated young patients will be of great interest.

The small blood vessels of the interstitium sometimes showed deposits of a refractile acidophilic material beneath the apparently intact endothelium, with considerable restriction of the lumen (Fig. 100).

Even before there was a marked shrinkage of the tubules there might be a suggestive increase in the interstitial cells of Leydig not resulting from compaction of the tissue. This occurred uncommonly but is evident in Figure 101. It is apparently the result of hyperplasia of the Leydig cells rather than of compaction.

Changes in the hypophysis, presumably associated with the lesions in the testes, will be discussed later in this report.



*Ovaries and Uterus (Table XXVII).* Little suitable material from ovaries and uterus was available for inspection. There were sections of the ovaries from 10 of the 12 female patients in group II. Of these, 3 were of postmenopausal and 2 of premenstrual age. The latter usually showed numerous undeveloped follicles, some in process of atresia. In

TABLE XXVII  
*Hiroshima, Group II: Ovaries and Uterus*

Observations	Sub-group		
	211	212	222
Ovaries			
Histologic specimens available	10	0	0
Postmenopausal	3		
Pre-menarche	2		
Ova and corpora albicantia present, developing follicles absent	4		
Marked atresia of follicles*	1		
Corpus luteum of pregnancy	1		
Uterus			
Histologic specimens available	6	0	0
Endometrium in resting phase*	3		
Postmenopausal	2		
Decidual reaction, following pregnancy	1		

\* In sexually mature women.

the former there was complete involution. The four ovaries from non-pregnant women in the functional age group had in common the presence of corpora albicantia and primordial follicles without developing follicles or corpora lutea. In one young woman the primary follicles were remarkably few and those that remained were in process of atresia (Fig. 109). In some instances the ova had become shrunken in this case (Fig. 111) and granulosa cells compacted in a group at the center of the follicle. Subendothelial acidophilic deposits restricted the lumina of some vessels of the ovary as of the testis (Fig. 112).

The endometrium in all of these cases was in the "resting phase" and the central glands were lined by columnar epithelium that showed no evidence of corpus luteum effect. The spindle-shaped cells of the endometrial stroma were thin, small, and elongated.

One pregnant patient, 38 years of age (K-61) who had been in a Japanese building at 1100 yds. and who had subsequently become epilated, died on the 35th day apparently of ulcerative enteritis, shortly after she had aborted a 5 months' fetus. A corpus luteum of pregnancy was found in an ovary and there were still fragments of decidual tissue in the uterus. The resistance of the corpus luteum of pregnancy to irradiation is well known.

Neither the uterus nor its appendages escaped the hemorrhages that

involved the other tissues. They occurred in the endometrium or serosa of the uterus, the ovaries, and fallopian tubes (Fig. 108).

*Brain (Table XXVIII).* Congestion of the leptomeninges and intracerebral vessels usually was in evidence. In one instance there were large subpial, or multiple punctate subependymal hemorrhages (Fig. 113).

TABLE XXVIII  
*Hiroshima, Group II: Brain*

Observations	Sub-group		
	211	212	222
Gross specimens available	37	0	0
Histologic specimens available	7	0	0
Subpial hemorrhages	5		
Subependymal petechiae	1		
Petechiae of cerebral substance	1		
Ecchymosis of dura	1		
Epidural hematoma	1		
Focal hemorrhage and necrosis	1		

Petechiae or ring hemorrhages were found also in several sites, including the corpus callosum, within the same brain. In another patient masses of bacteria were found within necrotic tissue surrounded by hemorrhage (Fig. 114). About these there was no leukocytic response.

There was evidence of former trauma in one epidural hematoma, but most of the hemorrhagic lesions probably had the same pathogenesis as those of the other organs.

The ganglion cells in general were well preserved, except in the vicinity of foci of hemorrhage or necrosis.

*Adrenals (Table XXIX).* The adrenals were almost always remarkable for their small size, which bore no apparent relation to the nutri-

TABLE XXIX  
*Hiroshima, Group II: Adrenals*

Observations	Sub-group		
	211	212	222
Gross specimens available	58	5	2
Histologic specimens available	47	4	1
Gross evidence of loss of lipoid, no microscopic sections available	11		1
Atrophy of cortex, especially of the zona glomerulosa	30	2	
Focal necrosis	1	1	
Fibrinous material in periadrenal fat	3		
Periadrenal hemorrhages	8	1	
Hemorrhages of cortex	3	1	
Heavy plasma cell infiltration	1		
Thrombus in arteriole		1	

tional state of the patient. The outer cortex usually was remarkably narrow and was composed of translucent yellow-gray, rather than

orange-yellow, opaque tissue. This change usually was diffuse, but might be focal. The cortex at times came to resemble the gray, translucent, medullary substance. In a few instances cortical or periadrenal hemorrhages might be seen but in no instance was there a notable degree of disruption of the organ. Often, in association with the hemorrhage, there had been exudation of protein-containing material or fibrin into the areolar septa of the periadrenal fat.

Microscopically, the cells of all layers usually had finely granular rather than vacuolated cytoplasm, confirming the impression of loss of cortical lipid that was gained from the gross inspection. The cells of the deeper fascicular and reticular layers tended in some measure to retain their vacuolation. In some instances, scattered groups of cells, especially in the zona fasciculata, had extremely foamy cytoplasm and pyknotic, centrally placed nuclei. In one such, there was also focal necrosis with infiltration of large fat-filled phagocytes (Fig. 117). A remarkable finding in the same case was that of numerous mitotic figures in the epithelial cells of the central part of the zona fasciculata (Fig. 118).

Cells resembling plasma cells usually were present in small numbers in the medulla, at the corticomedullary junction, and at times also in the cortex. In one instance these cells were very numerous for no evident reason. There was no indication that they represented erythropoietic or myelopoietic tissues.

No information is available concerning the effects of castration upon the adrenals in man. In male mice, however, an increase in the thickness of the zona reticularis has been observed.<sup>56</sup> The pathogenesis of the adrenal atrophy observed in many of group II patients is unknown.

*Thyroid Gland.* Fifty-four gross and 16 histologic specimens of thyroid tissue were available. The follicles usually were small, lined by low-cuboidal epithelium, and filled with palely staining colloid.

*Thymus.* There were no notable changes in the 3 available specimens of thymus except for hemorrhage in one.

*Pituitary Body.* Twenty hypophyses were available in which the cells were well enough preserved to be recognizable as to type. Four were from female and 16 from male patients. Fixation, however, was imperfect in most of these since the capsule of the gland usually had not been opened before it had been put in the formalin solution.

Large basophilic vacuolated "castration cells" were found in 4 of the 16 male patients. In the instance illustrated in Figure 37, the basophilic cells generally were large and some had huge vesicular nuclei. In many cells vacuoles had formed in the cytoplasm and the basophilic granules were widely scattered within the trabeculae of cytoplasm. Vacuolization

was extreme in some cells which had become gigantic. The basophilic granules in them were few and the nucleus was displaced to one side in "signet ring" fashion. Cells of this type are found in most species of animals following castration.<sup>77</sup>

Some coarsely vacuolated basophils were found also in one female patient who had shortly before aborted a 5 months' fetus. Groups of large "pregnancy cells" were present also in this hypophysis.

In one other male and in one female patient the basophilic cells were thought to be unusually large and numerous although they were not vacuolated. However, it is difficult to draw conclusions from these sections without statistically adequate measurements and counts. Unfortunately, only single slides are available.

An interesting incidental finding in one case (K-38) was the presence of a minute chromophobe adenoma.

It is of interest to note that these changes in the pituitary body took place despite the fact that the Leydig cells remained morphologically intact and were numerous following complete atrophy of the germinal epithelium of the testes.

*Neck and Mouth Organs (Table XXX).* Hemorrhagic and necrotizing lesions of the mouth and neck organs were frequent at this stage,

TABLE XXX  
*Hiroshima, Group II: Mouth and Neck Organs*

Observations	Sub-group		
	211	212	222
Gross specimens available	53	5	2
Gingivitis, all types	23		
Hemorrhagic	12		
Necrotizing	9		
Type unstated	2		
Necrotizing glossitis	3		1
Necrotizing tonsillitis	65		
Faucial	40	1	2
Lingual	25		
Necrotizing pharyngitis	13		
Necrotizing epiglottitis	21		
Hemorrhages of epiglottis	4		
Edema of epiglottis	3		
Necrotizing laryngitis	7		
Hemorrhages of larynx	4		
Edema of larynx	2		
Necrotizing tracheitis	1		
Hemorrhage of trachea		1	
Ulcerative esophagitis	2		
Petechiae of esophagus	1		
Leukoplakia of esophagus	1		

especially in the gums and tonsils. They appeared at a time when leukopenia had made the patients most susceptible to infection. What rôle

such direct radiation effects as have been described in the patients of group I play in their causation is problematic.

The gingivae became greatly swollen and infiltrated with extravasated blood. Their margins became necrotic, often with much sloughing of tissue, so that the alveolar process was exposed. Nevertheless, the teeth remained firmly attached. Pressure released quantities of hemorrhagic material from the periodontal tissues. Healing might occur with a superficial scar, resembling a zone of leukoplakia upon gross inspection. The lips as well as the lining of the mouth might become similarly involved.

Occasionally, the anterior portion of the tongue was the seat of necrosis and hemorrhage (Fig. 124), but these changes were more frequent on the dorsum where they involved the covering epithelium as well as the substance of the subjacent lymphoid follicles (Fig. 123).

More common than lingual was faucial tonsillitis. The tonsils usually were moderately and sometimes greatly enlarged, but on occasion there was no increase in size, despite total necrosis of the organ (Fig. 122). There often was complete necrosis also of tissue elsewhere in the wall of the pharynx, apart from the lymphatic tissue. Such lesions often were outlined by hemorrhagic borders and were seen also in the epiglottis, larynx, and trachea (Fig. 122).

Histologically, these lesions had in common a uniform necrosis with no barrier of polymorphonuclear leukocytes (Fig. 125) although there was a sharp line of demarcation from the relatively intact tissue. Beneath and about the lesion, however, there frequently was edema and sometimes hemorrhage. The cellular exudate, when present, consisted of scattered plasma cells and occasional small and large mononuclear elements (Fig. 126). Often, as in the tonsils, the necrosis involved lymphoid tissue, whose mature lymphocytes, as elsewhere, had almost completely disappeared and in which there was compaction of the reticulum. The reticulum had in many cases differentiated into the large atypical cells that have been described previously. In one case the lesions of thrush were observed (Fig. 127), suggesting the low state of resistance of the patient.

*Skin.* Of 65 patients in group II from whom specimens of skin were available, 58 were noted to have cutaneous hemorrhages at the time of death (Table XXXI). These varied from petechiae (Fig. 128) to large ecchymoses.

Ulcerative lesions of the skin not related to burns also were frequent and several were pustular. An example of an ulcerative lesion of a common type is shown in Figures 129, 131, and 132. As the region of

TABLE XXXI  
Hiroshima, Group II: Skin

Observations	Sub-group		
	211	212	222
Gross specimens available	58	5	2
Epilation			
Scalp	48	2	
Axillary	8		
Pubic	6		
Eyebrows	4	1	
Beard	2		
Petechiae or purpura	53	4	1
Ulcers	20		
Pustules	3		1

denudation of the epithelium was approached, the superficial layers of cells became swollen and frequently the nuclei were found as contracted, deeply staining masses, situated within a clear cytoplasm. The deeper epithelial layers were well preserved and their nuclei were large and finely dotted with chromatin. Pigment also was present within the cells. As the margin of the ulcer was approached, the epithelium became more and more vacuolated and ultimately the nucleus was lost. In some places there was parakeratosis. The base of the ulcer itself consisted of necrotic collagen which was focally infiltrated with numerous erythrocytes. There were no leukocytes except in the septa of areolar tissue that traversed the deeper layers of the subcutaneous fat. These were of small mononuclear and plasma cell types. Even at a distance from the ulcer the skin appendages were surrounded by groups of small mononuclear cells and plasma cells. So also were the vessels of the papillae. In another instance (Fig. 130) exudate was more abundant, but it consisted largely of plasma cells and large mononuclear elements.

The mechanism of origin of the ulcerative lesions is of interest. Whether they were merely the result of infection of the skin in the patients with leukopenia or whether they were in part also the direct result of radiation is difficult to decide. The Japanese described the appearance of "blisters" of the unburned skin of some of the exposed patients. In the records of the necropsies of 2 individuals, K-98 (group I) and K-109 (group II), who were recently dead, the skin was said to have "peeled" easily revealing a pink raw surface beneath. The tongue, pharynx, and esophagus of one of these patients, K-98, showed remarkable changes in the epithelium with sloughing over large areas. This has already been described (Figs. 119 to 121). This patient died on the tenth day after exposure. Unfortunately, skin was not available and there is no supporting evidence for the concept that such ulcerative lesions are direct radiation effects in patients who died at a later date.

The only unburned skin available from most of these patients was the scalp. The changes in the surface epithelium of that region were slight, and in the patients dying later there was no evidence of telangiectatic lesions nor of any remarkable alterations in the collagen.

Epilation appeared in most instances approximately 2 weeks after the irradiation and involved men and women alike. It tended to have the same distribution as ordinary baldness in men, but in some instances the temporal and occipital regions also became epilated. Even then, a few hairs tended to remain (Fig. 9). The beard and eyebrows, and the axillary and pubic hair were relatively resistant to epilation. Regrowth of the hair usually was in progress within 8 weeks after the bombing at Hiroshima and at about 12 weeks at Nagasaki, and the new hair, initially finer, ultimately possessed the same color and texture as the old (Fig. 133).

The changes in the hair follicles are of interest and were illustrated in all phases in heavily radiated patients. If a typical hair follicle is examined (Fig. 134), it will be seen that from the matrix at the root there are differentiated strata of acidophilic trichohyalin-bearing cells (Henle's and Huxley's layers) forming an internal root sheath which is separated along a cuticle from the external root sheath. The latter is an invagination of the epithelium of the surface. Keratinization of the hair begins internally, from the trichohyalin-bearing cells of the internal root sheath very near the base of the follicle. A large keratinized shaft of hair consequently passes through the corium on its way to the surface, separated for much of its course from the external root sheath by a space.

In follicles of the scalp of the irradiated patients, however, the trichohyaline layer failed to differentiate, so that a plug of epithelium resulted which had the structure of, and was continuous with, the external root sheath. Moreover, the pigment was found irregularly distributed throughout the epithelium even at a distance from the papilla (Fig. 135). The epithelium of the matrix became flattened and less basophilic and mitotic figures among its cells became rare, whereas usually they were numerous. The matrix then formed a shallow cap rather than a long hood over the tip of the papilla. The papilla itself became less vascular and smaller and its component elongated spindle-shaped cells were compacted. Often the papilla ceased to be intimately applied to the matrix epithelium and a space appeared between the two tissues. The plug of epithelium at the base of the follicle became more and more narrow, and a whorled, pearl-like arrangement of cells might result (Fig. 136). At the same time both the glassy sheath and the external, more cellular,

fibrous membrane became considerably and often greatly thickened. Spaces appeared beneath the glassy sheath and the shrinking plug of epithelium which had become the core of the follicle. Stains for elastic tissue demonstrated that, despite its wavy refractile character, the glassy membrane was composed of collagenous rather than elastic tissue (Fig. 137). The immense thickening of the basement membranes that might occur in every follicle is demonstrated in Figure 138.

With the failure of formation of the internal root sheath, the hair shaft apparently took origin directly from the external root sheath by a process analogous to that of parakeratosis. The point of origin of the shaft came closer and closer to the surface as the matrix substance atrophied, and ultimately there remained only a bulbous plug of parakeratotic material near the mouth of the follicle (Fig. 139). This process was reflected in the structure of the cast-off hair (Fig. 140), which tapered near its formerly attached end and which was devoid of the remnants of the internal root sheath that usually adhere as a cuff.

The follicles, despite these changes, did not lose their capacity for regeneration, as shown in scalps from patients of group III (dying after the sixth week). The process of regeneration and the analogy with the normal processes of replacement of hair are described subsequently.

Other changes in the scalp were inconspicuous. There might be atrophy of the rete pegs and a general thinning of the epithelium at the surface, sometimes associated with hyperkeratosis (Fig. 138). The keratotic material was particularly abundant in the mouths of the hair follicles. Hyperpigmentation sometimes was observed in the scalps of these epilated patients. The pigment was situated in the basal layer of the epithelial cells. Atrophy of the sebaceous glands frequently was observed (Fig. 139). This process went hand in hand with atrophy of the hair follicle and cannot be interpreted as a direct radiation effect. The sweat glands in skin that had not been burned showed relatively little change, although occasionally the acini appeared shrunken and had thickened basement membranes and tiny vacuolated epithelial cells with pyknotic nuclei.

#### *Group III: Patients Dying after the Sixth Week*

The patients of group III, those dying after the sixth week, as a rule showed the effects of malnutrition. By that time the bone marrow usually had recovered or had become hyperplastic, and there was evidence of the delivery of leukocytes to the lesions in the tissue. In occasional instances, however, there was evidence of a maturation



defect, and in a few patients the marrow remained hypoplastic. Concomitantly with the recovery of the marrow, hemorrhagic lesions were relatively rare despite the necrosis of some tissues.

The important lesions at the time of death were focal necrotizing pneumonia (sometimes in process of organization) and necrotizing enterocolitis. In both of these, polymorphonuclear leukocytes usually were now prominent elements of the exudate. It is probable that the depression of hemopoiesis that occurred for a time in many of these patients was the important factor in permitting these infections to gain headway. Stigmata of radiation effect, particularly epilation and testicular atrophy, were still present, but in some patients considerable regrowth of hair had occurred that was manifest in regenerative processes histologically.

Tables XXXII to XLVI include data for group III patients from Nagasaki, since in this group information regarding distance from the bomb was stated in the protocols.

The clinical data pertinent to the group III patients who were necropsied are given in Table XVI.

*Heart (Table XXXII).* Aside from hemorrhages and the apparently adventitious lesions regarded as "rheumatic," and for one instance each in which gross diagnoses of "fatty change" and "focal necrosis" were substantiated microscopically, there were no significant changes in the heart.

*Lungs (Table XXXIII).* Multiple pulmonary hemorrhages were noted in one instance. In most cases there were necrotizing lesions of the lungs heavily infiltrated with polymorphonuclear leukocytes. In two of these (Figs. 25 to 27) abscesses had formed, with fibrous walls surrounded by large zones of organizing pneumonia. In 3 patients there again was evidence of "neutropenic necrotizing pneumonia." The marrow of one of these, who had been heavily exposed to radiation, exhibited a very remarkable degree of hyperplasia and in the other cases the marrow was aplastic. Three patients died of advanced fibrocaseous pulmonary tuberculosis.

*Spleen (Table XXXIV).* In the spleen, the lymphoid tissue usually was still atrophic and atypical large mononuclear cells tended to persist, but there was evidence of recovery in some cases. In these there were now abundant lymphocytes about the central vessels of the malpighian corpuscles, and in many of them germinal centers had begun to appear (Fig. 48). In certain of the corpuscles of such patients a few groups of large reticulum cells were still discernible in compact arrangement at the periphery of the collars of mature lymphocytes.

TABLE XXXII  
*Group III: Heart*

Observations	Hiroshima				Nagasaki			
	311	Sub-group			311	Sub-group		
		312	321	322		312	321	322
Gross specimens available	9	1	4	3	6	3	1	6
Epicardial hemorrhages	4				2	1		
Endocardial hemorrhages	1							
Fatty change of myocardium	1							1
Focal necrosis of myocardium								1
Additional observations								
Fibrous pericardial adhesions	1				1			
Chronic verrucous mitral endocarditis	1			2				
Chronic verrucous tricuspid, mitral, and aortic endocarditis							1	

TABLE XXXIII  
*Group III: Lungs*

Observations	Hiroshima				Nagasaki			
	311	Sub-group			311	Sub-group		
		312	321	322		312	321	322
Histologic specimens available	9	1	4	3	6	3	1	6
Focal atelectasis and emphysema			1	1				
Multiple hemorrhages							1	
Focal neutropenic pneumonia	2		1					
Focal necrotizing pneumonia with polymorphonuclear cells, all	6							
Same (with organization)	2				4	2		3
Fibrocaceous pulmonary tuberculosis				2				2
Fibrinous pleurisy	1							1
Additional diagnoses								
Fibrous pleural adhesions				2	1		1	1
Healed tuberculous foci	1		1					
Interstitial pulmonary fibrosis		1						
Tuberculosis of tracheobronchial lymph nodes						1		
Hydrothorax								1

TABLE XXXIV  
*Group III: Spleen*

Observations	Hiroshima				Nagasaki			
	311	Sub-group			311	Sub-group		
		312	321	322		312	321	322
Histologic specimens available	9	1	4	2	6	3	1	5
Perisplenic adhesions	2							
Decrease of lymphocytes	6		3		5	2		2
Many lymphocytes, no germinal centers	1		1	1				1
Absence of germinal centers	7		4	1	5	1		2
Germinal centers present	2	1		1	1	2	1	2
Atypical mononuclear cells	6				3	1		1
Heavy plasma cell infiltration	1							
Focal necrosis (bacterial emboli)			1					
Infarct						1		
Hemorrhage in capsule							1	
Tuberculosis								1

*Lymph Nodes (Table XXXV).* The lymph nodes appeared to be even more laggard than the spleen in regard to restoration of germinal centers. They were present in the nodes of only one patient. Most lymph nodes still manifested loss of mature lymphocytes, compaction of reticulum and atypical large cells as previously described (Fig. 54).

TABLE XXXV  
*Group III: Lymph Nodes*

Observations	Hiroshima				Nagasaki			
	311	312	321	322	311	312	321	322
Histologic specimens available	6	0	1	1	5	1	1	1
Decrease of lymphocytes	5		1		5		1	1
Lymph nodes composed of typical lymphocytes, but without germinal centers				1				
Absence of germinal centers	5		1	1	4		1	1
Germinal centers present	1				1			
Atypical mononuclear cells	6		1		2			
Additional diagnoses								
Tuberculosis of tracheobronchial or hilar nodes					1	1		

*Bone Marrow (Table XXXVI).* Although the marrow might remain hypoplastic, there was usually at least focal regeneration (Figs. 69 and 70), especially in the myeloid series, and sometimes marked myeloid hyperplasia (Figs. 57 and 72). This was manifest in the long bones as well as in the ribs, sternum, and vertebrae. Most patients dying at this stage had a severe anemia that might in part be associated with the omnipresent severe infections.

In some of the marrows from Nagasaki patients, relatively large numbers of eosinophilic myelocytes existed.

A typical example of a group III marrow was found in K-50. In a section of vertebrae, the marrow appeared to be hyperplastic. The cells were supported within congested and even hemorrhagic tissue. The most numerous elements in the cellular population appeared to be the younger neutrophilic and eosinophilic myelocytes. There were relatively few late myelocytes, band cells, and mature polymorphonuclear leukocytes. Occasional blast forms were seen and some cells were in mitosis. There also were many megakaryocytes and numerous small islands of erythropoietic tissue. Fairly large numbers of plasma cells, some multinucleated, also were in evidence, together with lymphocytes and large stellate phagocytes filled with hemosiderin.

In sections of rib there was still an abundance of "gelatinous marrow," within which were islands of hemopoietic tissue, again containing a predominance of younger forms (Fig. 70). The tissue was essentially

similar to the vertebral marrow except for the relatively large amounts of acellular material.

The upper half of the shaft of a femur of this patient had a remarkable gross appearance (Fig. 34). The abundant marrow contained among the stout trabeculae of bone had a translucent currant-jelly appearance in

TABLE XXXVI  
*Group III: Bone Marrow*

Observations	Hiroshima				Nagasaki*			
	311	Sub-group			311	Sub-group		
		312	321	322		312	321	322
Long bones								
Histologic specimens available	6	1	3	3	1	1	1	1
Type A: hypoplasia	1		2			1		
Type B: marked focal reticulum hyperplasia				2				1
Type C: focal myeloid regeneration	4	1	1		1		1	
Type D: marked myeloid hyperplasia	1			1				
Flat bones								
Histologic specimens available	3	0	0	0	5	2	1	3
Type A: hypoplasia								
Type B: marked focal reticulum hyperplasia	2				1			
Type C: focal myeloid regeneration	1				4	2	1	2
Type D: marked myeloid hyperplasia								1
No bone marrow								
Polymorphonuclear cells in tissues			1					
Additional diagnosis								
Caseous tuberculosis of marrow								1

\* The case of monocytic leukemia is not tabulated with the others.

part, but this tissue contained foci of much more opaque gray-red or pale red-brown substance. This was yielded in large friable fragments to the knife. In smears of this tissue there were clumps of young cells with coarsely reticular nuclei and abundant basophilic cytoplasm. These often were associated with islands of normoblasts and were identified as basophilic erythroblasts. There also were myelocytes and scattered plasma cells within an abundant "gelatinous" matrix.

Smear preparations of these various tissues stained by the Wright-Giemsa method were excellently preserved (Fig. 71). The only unusual feature was the presence of relatively large numbers of lymphocytes and of plasma cells, some of which were multinucleated. The granules of the myelocytes were well stained everywhere, even in the youngest forms. There were some cells resembling myelocytes, but with clear cytoplasm. These might be forms intermediate between the reticular elements and the myelocytes. Band cells and a few polymorphonuclear leukocytes also were found.

The background substance in "gelatinous marrow," as seen in K-50, deserves comment. It had a delicately fibrillar structure, which was apparent in the hematoxylin and eosin preparation. In Masson and phosphotungstic acid hematoxylin stains, this material gave the reaction of fibrin (Fig. 74). It was deposited most densely about cells, some of which had the structure of megakaryocytes. Smaller elements, more like reticulum cells, and some cells intermediate between these and megakaryocytes also acted as centers upon which fibrin was deposited (Fig. 73).

**Relation to Peripheral Leukocyte Count.** Relatively few patients who lived beyond the sixth week exhibited a persistent leukopenia. In patients who did not develop a leukocytosis in response to an acute infection, the bone marrow at necropsy showed either evidence of maturation defect and was hyperplastic, as in the case which has just been described, or the marrow showed varying degrees of hypoplasia. In K-50, despite the hyperplasia of the marrow demonstrated in Figures 34 and 70, the peripheral white blood cell counts did not exceed 6500.

*Laboratory Data—K-50*

	Date of examination of blood			
	Sept. 19	Oct. 8	Oct. 15	Nov. 8
Red blood cells ( <i>millions</i> )	2.2	1.5	1.8	1.7
Hemoglobin ( <i>per cent</i> )	36	31	62	35
White blood cells	3200	5100	6500	4300
Polymorphonuclear cells, mature ( <i>per cent</i> )			47.5	8
Band cells ( <i>per cent</i> )			25	53
Metamyelocytes ( <i>per cent</i> )			6	8
Myelocytes ( <i>per cent</i> )			0	5
Lymphocytes ( <i>per cent</i> )			13.5	14
Monocytes ( <i>per cent</i> )			8	7
Eosinophils ( <i>per cent</i> )			0	3
Basophils ( <i>per cent</i> )			0	2
Reticulocytes (Oct. 15, 1945): 18 per cent				
Platelets (Oct. 15, 1945): 45,900 per cmm.				
Protein (CuSO <sub>4</sub> method): 6.6 gm. per cent				

Notable features of the counts were the anemia, and the shift to the left in the granulocytic series. A large number of reticulocytes also was observed, indicating a regenerating but as yet insufficient erythropoietic tissue. This patient died on November 15, 1945, of a necrotizing and organizing pneumonia (Figs. 25 and 27).

In most patients surviving into the third period, there was leukocytosis associated with hyperplasia of the bone marrow, despite previous leukopenia. K-118 (sub-group 311) is an example.

*Clinical History—K-118*

K-118 was a 56-year-old woman who was at 700 yds. She was thrown a distance by the blast and sustained slight contusions of the back and the legs. At the time

of the bombing she was out of doors cutting grass close to a wooden Japanese building. Few observations were recorded in the clinical history. There was nausea but the date of its occurrence was not stated. On August 26 diarrhea appeared which persisted to the time of death. It was of sanguineous type. Epilation was present but the date of onset was not stated, and there was also pigmentation of the skin. The patient was unconscious shortly after the blast and was carried to the village of Izumite where she regained consciousness. During the entire course she had only a slight fever. She died on October 5, 1945.

	<i>Red blood cells</i>	<i>White blood cells</i>
August 29	2.44 millions	1050
September 1	1.74 millions	570
September 7	3.30 millions	1040
September 15	5.31 millions	2900
September 25	3.92 millions	3600
September 29	3.72 millions	11,400
October 1	3.85 millions	17,700

The important lesions at necropsy were hemorrhagic pneumonia and ulcerative ileocolitis.

The bone marrow showed reticulum hyperplasia (type B), as illustrated in Figure 69, and there was considerable differentiation into myeloid tissue, but numerous plasma cells were still present. In the lymph nodes of this patient there was lymphoblastic hyperplasia but there was no evidence of leukemic infiltration of the tissues.

In some instances, myeloid hyperplasia in patients dying in this period was extreme and the adipose tissue of the marrow had almost completely disappeared (K-14, Fig. 72). The marrow in this case was of type D. This patient had a moderate leukocytosis before his death from acute suppurative renal disease on September 22, 1945.

A single instance of leukemia was encountered during the study of some 14,000 patients in both cities. Although this case is considered purely adventitious, it is presented in detail for the reason that the patient had been close to the bomb and had had a striking leukopenia before the leukemia developed.

#### *Case Report\*; M. Matsuo, Autopsy K-224*

The patient was a 19-year-old schoolboy, who was at a munitions factory in Nagasaki, approximately 1000 yds. removed from the center. He lost consciousness and sustained a slight burn of the right leg at the bombing. A few days later he suffered from anorexia but gradually recovered. On August 28, epilation appeared and on September 3 there was fever accompanying the onset of petechiae and sore throat.

He was admitted to the hospital on September 8, when there were petechiae over the whole body, particularly upon the abdomen. The pharynx was congested but

\* Abstracted, in part, from "Report of a Case of Monocytic Leukemia Occurring Following the 'Atomic Bomb Disease'" by Dr. Tando Misao, Dr. Yoshimichi Harada, and Dr. K. Hattori, Faculty of Medicine, Kyushu Imperial University.

there was no necrosis. At that time there was a marked decrease in the red blood cell count although the hemoglobin was recorded as "100 per cent." There was marked leukopenia and the sedimentation rate was increased. The nucleated cells of the marrow were found to be 22,600 per cmm. and there was an excess of lymphocytes and plasma cells. Vitamin B and liver extract were administered and the bones were irradiated with ultraviolet light. The patient improved, the white blood cell count reaching 5500 shortly before his discharge on September 30, 1945. His red blood cell count was then 3.29 millions with 77 per cent hemoglobin.

He returned to school after leaving the hospital and had no complaints. For 3 days after November 4 he worked hard at the rice harvest, but complained of a tired feeling. On the evening of November 6 there was a sensation of fever accompanied by headache, swelling of the gums with bleeding, pharyngitis with pain in the throat and difficulty in swallowing. The next day he had a high fever and there were petechiae of the extremities. On November 9 he had some sanguineous stools and was readmitted to the hospital on November 12. At that time his pulse rate was elevated to 120. There was edema and pallor of the face. Petechiae were seen everywhere in the skin and on the buccal mucous membrane. The gums were swollen. The soft palate, particularly on the right side, was remarkably swollen and was partly covered with a thick white membrane. There was gangrene of the right tonsil. Erosions were observed on the nasal mucous membrane and there was hemorrhage from the drum of the left ear. Hemorrhages were seen also on each retina. The cervical lymph nodes were enlarged to the size of a thumb and several axillary nodes of this size were palpated also. Both the liver and spleen were found to be slightly below the costal margin upon clinical examination.

Laboratory examinations showed a few red blood cells in the urine. The stools were tarry and diarrheal, and were strongly positive for blood. Hematologic examination showed a white blood cell count of 390,000 with a predominance of cells thought to be monocytes (Fig. 75). Eighty-one per cent of these cells phagocytized carbon in living preparations (Fig. 76). Two and one-half per cent of all cells were positive by the peroxidase reaction and 0.9 per cent of the monocytes were positive. The nucleated cells of the marrow numbered 566,400 per cmm., of which 91.2 per cent consisted of monocytes, including many young forms. A few of these cells were in mitosis and some vacuolated forms were found also. The hepatic and splenic punctures showed cells of similar type.

Course in Hospital. His temperature was sustained at 39° to 40° C. and the pulse rate was 100 to 120. He was given 1000 cc. of Ringer's solution, vitamins B and C, "cardiac tonics," and gargles. At approximately 1:30 a.m. on November 16, dyspnea appeared, the respiratory rate being 44 to 48, there was tachycardia of 140 to 160, and he died a few hours later in collapse.

The important laboratory and temperature data are summarized in Text-Figure 2.

#### *Findings at Necropsy: Gross Notes*

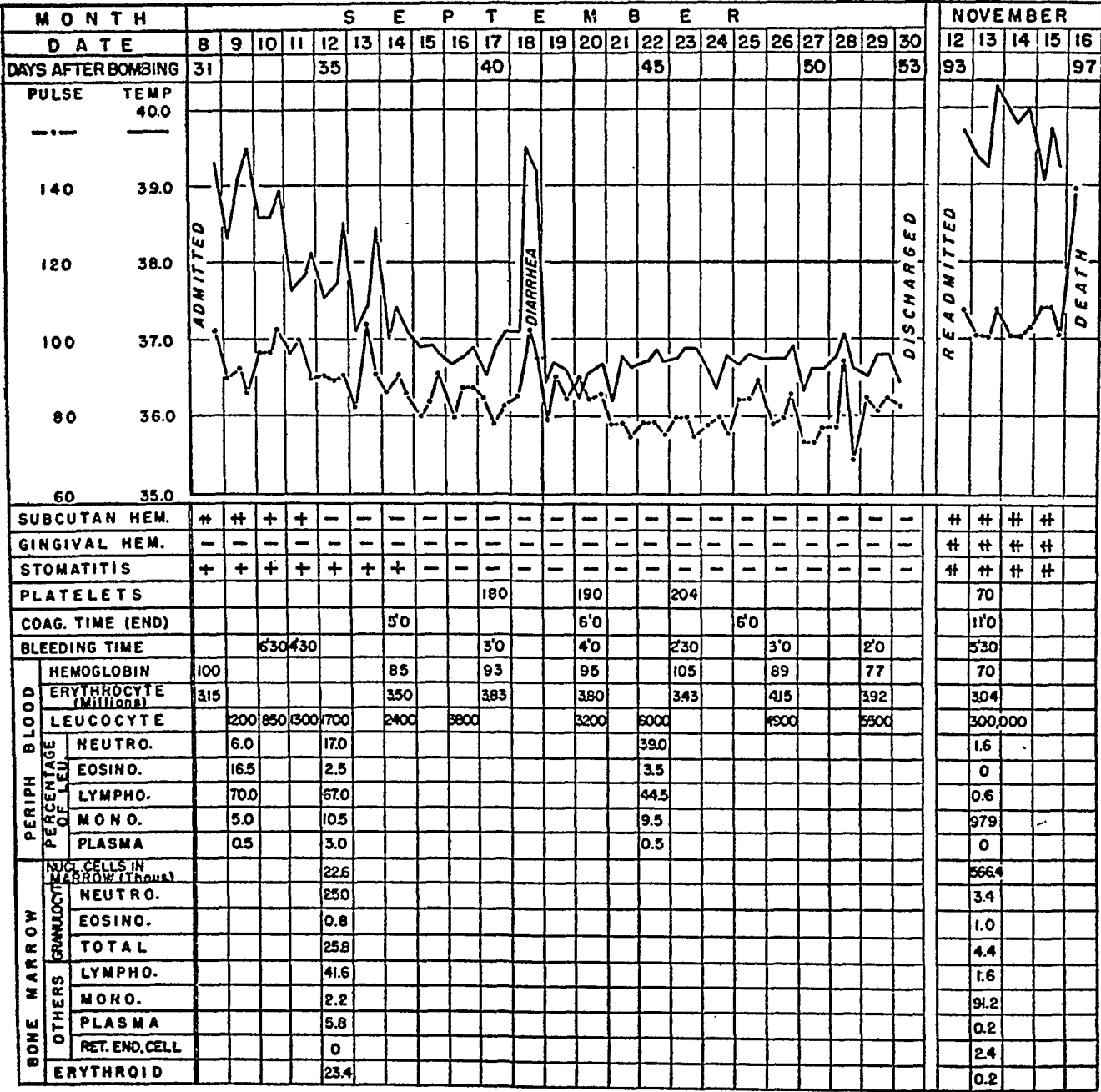
There were petechiae of the skin, pericardium, pleura, peritoneum, Glisson's capsule, renal pelves, pharynx, esophagus, large intestine, and submucosa of the oral cavity. Approximately 100 cc. of fluid blood were found in the peritoneal cavity. Small foci of hemorrhage were seen in the pulmonary parenchyma. There was necrosis of the right tonsil and a gray membrane involved both tonsillar regions and the soft palate. On the mitral valve a few verrucous masses were seen. Focal pneumonia was found in the right lower lobe. The spleen weighed 230 gm. and on the cut surface had an irregularly mottled, dark red appearance. The trabeculae were indistinct. The lymph nodes of the cervical, mediastinal, and axillary regions were enlarged as were those of the stomach and intestines. Hemorrhagic erosions and small ulcers were seen throughout the gastro-intestinal tract. Pyramidal yellow-gray zones were found in the kidneys which were thought to be infarcts.

Histologic Notes

Large numbers of mononuclear cells were found throughout most of the tissues. The cells occurred in closely compacted groups and consequently some were polygonal in outline, and an occasional cell was spindle shaped. Most of the cells presented spherical or slightly indented nuclei with prominent nucleoli. The cytoplasm apparently was much less abundant than in the cells seen in the peripheral blood. In many places groups of the atypical cells had become necrotic.

Such mononuclear infiltrations were situated among the muscle fibers of the heart,

MONOCYTIC LEUKEMIA\*



\* Chart compiled by: Prof. T. Misao, M.D., Y. Harada, M.D., K. Hattori, M.D., Fukuoka

Text-Figure 2



some of which consequently became atrophic. The small blood vessels of the interstitium also were filled with atypical cells.

The walls of the alveoli of the lungs were thickened by large numbers of the atypical mononuclear cells. There also was focal necrosis about the bronchioles, whose walls had become necrotic. Polymorphonuclear cells were not seen.

In the liver the pericentral sinusoids were especially involved and there were only occasional mononuclear cells in the periportal connective tissue (Fig. 79). The hepatic cell cords at their central ends had become strikingly atrophic.

In the intestines there were tremendous submucosal infiltrations but these were fewer in the mucosa. The latter, however, had become necrotic in many large foci.

In striated muscle (Fig. 78) there was massive infiltration of cells among the fibers, some of which had become atrophic. Occasionally one of the mononuclear elements was found in mitosis. Groups of cells had suffered necrosis, but for the most part the tissue was well preserved.

In the pharynx there were striking subepithelial infiltrations. The epithelium in the section available, however, appeared intact.

Large interstitial and perivascular infiltrations were found throughout the kidneys. Many cells of the mononuclear type also occupied a subcapsular position.

The lymph nodes consisted of solid masses of the mononuclear cells such as have been described previously. There were large foci of necrosis.

The small fragment of marrow (Fig. 77) that was available contained only a few large fat cells, but there were large septa composed of masses of mononuclear cells. These had the same structure as they had elsewhere in the tissues. No islands of erythropoietic or myelopoietic tissue of the usual type were seen.

The vessels of the brain contained large mononuclear cells in massive collections; there was no interstitial infiltration in this organ.

*Gastro-intestinal Tract (Table XXXVII).* Ulcerative lesions of the intestine, especially of the colon, occurred often in group III. They again tended to be superficial and to be covered with fibrin. Sometimes the foci

TABLE XXXVII  
Group III: Gastro-intestinal Tract

Observations	Hiroshima				Nagasaki			
	311	312	321	322	311	312	321	322
<b>Stomach</b>								
Gross specimens available	9	1	4	3	6	3	1	6
Acute ulcer	1							1
Petechiae	4		1	1		1	1	2
Plasma cell infiltration						1		
<b>Small intestine</b>								
Gross specimens available	9	1	4	3	6	3	1	6
Ulcerative enteritis	1		1		2			1
Petechiae	1						1	2
Intussusception of ileum				1				
<b>Large intestine</b>								
Gross specimens available	9	1	4	3	6	3	1	6
Petechiae	4		1			1	1	2
Ulcerative colitis	2	1	3	2	2	2		
<b>Additional diagnoses</b>								
Ascariasis	2				3		1	
Amebic colitis	1				1			
Strongyloidosis					1			

of necrosis bulged into the lumen despite destruction of the epithelium. Polymorphonuclear leukocytes might appear in the lesions. In at least one case, however, they contained relatively few of these cells despite the hyperplastic marrow, and the ulcers were histologically similar to those of the aplastic stage.

TABLE XXXVIII  
*Group III: Liver and Gallbladder*

Observations	Hiroshima				Nagasaki			
	311	312	321	322	311	312	321	322
Microscopic specimens available	9	1	4	3	6	3	1	6
Giant nuclei in pericentral hepatic cells	1			1				
Central congestion, all	3	1	1	1	2		1	1
Central congestion, with necrosis	1				1		1	
Edema of pericentral connective tissue	2	1						
Focal fatty change of liver:								
Periportal	1		3	1		2		2
Midzonal	3							1
Pericentral	2							1
Disseminated					1			
Focal necrosis of liver					2			
Additional diagnoses								
Calculi in gallbladder			1					
Chronic cholecystitis						1		
Ascaris in bile duct			1					
Calculus in choledochal duct			1					
Chronic cholangitis			1					
Tuberculosis of liver								1

An intussusception of the ileum and localized peritonitis occurred in one patient. One case of amebic colitis was found in each series in this group (Fig. 92).

*Liver (Table XXXVIII).* Fatty changes in the liver were much more common at this stage than in the preceding groups of patients. They probably were associated with malnutrition and other factors rather than radiation. The change usually was mid-zonal or periportal and sometimes involved as much or more than one-half of the lobule. Necrosis, apparently in association with central congestion, was in evidence in 3 instances (Fig. 95). The mechanism of the central congestion was not clear as there were no evidences of acute lesions of the myocardium in these patients, despite the verrucous endocarditis that was occasionally encountered, nor were there chronic lesions such as a significant degree of mitral stenosis to account for the congestion. However, complete and detailed gross descriptions of the heart and detailed clinical information were not available. Disseminated foci of necrosis were encountered in 2 instances.

*Pancreas.* No significant lesions were discovered in the pancreas. The acini frequently were small, perhaps in association with the mal-

nutrition, but otherwise the cells were typical in structure and there was no evidence of fibrosis.

*Kidneys (Table XXXIX).* The kidneys exhibited no specific lesions. In one case there were multiple abscesses and another showed pyelonephritis. Polymorphonuclear leukocytes were present in large numbers

TABLE XXXIX  
Group III: Kidney

Observations	Hiroshima				Nagasaki			
	311	312	321	322	311	312	321	322
Histologic specimens available	8	1	4	3	6	3	1	6
Hemorrhage of pelvis	2	1	1		1			
Cloudy swelling	3	1	1		1			2
Scars of kidney	3	1	1		1	1		1
Abscesses (with polymorphonuclear cells)	1							
Atypical small and large mononuclear cells in sinusoids	1							
Infarcts					1			
Acute pyelonephritis						1		
Additional diagnoses								
Leiomyoma of renal pyramid	1							
Calculus of pelvis					1			1
Tuberculosis								1

in these lesions. In one patient there were the large and small mononuclear cells within the corticomedullary sinusoids that have been described previously.

*Ureters and Bladder.* There were petechiae of the bladder in 2 cases of sub-group 311 and in one of sub-group 312.

*Testes (Table XL).* In this group, all members of which were malnourished, the testicular atrophy was much more complete than in the preceding group (Figs. 39 and 105). The basement membranes usually were thickened and the Sertoli cells might be shrunken. Within the old

TABLE XL  
Group III: Testes

Observations	Hiroshima				Nagasaki			
	311	312	321	322	311	312	321	322
Histologic specimens available*	5	1	3†	2	2	2	0	2
Atrophy of germinal epithelium and derivatives	5	1	1	2	2	2		2
Thickening of basement membranes of tubules	5	1	1	1	2	2		2
Hyaline changes of blood vessels	4			1				
Hyperplasia of interstitial tissue	3			1	1			
Infantile testis			1					
Atrophy of Leydig cells, with hyperpigmentation						1		

\* All are from malnourished patients.

† One patient, 83 years of age, showed active spermatogenesis.

basement membranes there appeared in a broad band a less cellular, delicately fibrillar connective tissue. Spermatogenic tissue had completely disappeared. The tubules had shrunk and occasionally were completely hyalinized. The small blood vessels often had brightly acidophilic deposits of material beneath the endothelium and muscular wall, as seen in the earlier stages. In many cases there was now, in the opinion of one of us (A. A. L.), definite hyperplasia of interstitial tissue.

*Prostate.* An 83-year-old man, K-66, who was reputedly at 1800 yds., had an adenocarcinoma of the prostate. The testes of this patient, despite his age, were among the very few that showed no evidence of atrophy. In K-50, an emaciated 31-year-old man dying on the 100th day, in whom the testes were extremely atrophic, the prostate also was remarkably small grossly, as were the acini and epithelial cells histologically (Fig. 107).

*Ovaries (Table XLI).* In 5 premenstrual females who were within 1500 yds., numerous primordial follicles were still present. Developing

TABLE XLI  
*Group III: Ovaries, Uterus, Fallopian Tubes, and Vagina*

Observations	Hiroshima				Nagasaki			
	311	Sub-group 312 321 322			311	Sub-group 312 321 322		
Ovaries								
Histologic specimens available	4	0	1	1	3	0	1	3
Postmenopausal	1		1				1	1
Premenarche	1			1	2			1
Developing follicles absent,* corpora albicantia present	2				1			1
Hemorrhages							1	
Uterus, fallopian tubes, and vagina								
Histologic specimens available	1	0	1†	0	1	1	0	1
Endometrium in resting phase	1				1			
Myomata			1					
Endometriosis						1		
Acute salpingitis						1		
Tuberculosis								1
Chronic vaginitis								1

\* In premenopausal mature women.

† Postmenopausal.

follicles were absent in 3 in whom both corpora albicantia and primordial follicles were in evidence. Atrophy was much less evident than in the male.

*Brain (Table XLII).* Suppurative complications in the brain were relatively frequent. In this group there were 2 patients with suppurative meningitis and one with a "cerebral abscess." The abscess might possibly have been a tuberculous lesion since the patient had caseating pulmonary tuberculosis, but histologic sections were not available.

TABLE XLII  
Group III: Brain

Observations	Hiroshima				Nagasaki			
	311	312	321	322	311	312	321	322
Gross specimens available	8	1	4	3	6	2	1	6
Histologic specimens available	5	0	3	1	1	0	0	0
Petechiae of cerebrum	1		1					
Suppurative meningitis with polymorphonuclear cells	2							
Abscess of occipital lobe*				1				
Thrombus in superior longitudinal sinus	1							
Additional diagnoses								
Senile plaques			1					
Microgyria			1					
Cystic change of lenticular nucleus			1					

\* Gross specimen only.

*Adrenals (Table XLIII).* The atrophy that has been noted previously in the adrenal glands was extreme and in some cases involved all layers (Fig. 115). Usually the outer portion of the zona glomerulosa showed the most striking changes. Again it must be emphasized that most of these patients were emaciated.

TABLE XLIII  
Group III: Adrenals

Observations	Hiroshima				Nagasaki			
	311	312	321	322	311	312	321	322
Gross specimens available	9	1	4	3	5	3	1	5
Histologic specimens available	8	1	3	3	5	3	1	5
Gross evidence of loss of lipid, no microscopic sections available			1					
Atrophy of cortex, especially of the zona glomerulosa	6	1		1	5	2	1	5
Focal fatty changes of cortical epithelium			1		1	1		
Focal necrosis of cortical epithelium			1			1		
Periadrenal hemorrhages		1					1	
Hemorrhages of cortex	1		1				1	
Chronic passive congestion	2							
Additional diagnosis								
Tuberculosis						1		1

*Thyroid Gland (Table XLIV).* Occasionally in these emaciated patients the thyroid showed variation in the size of the follicles. In such thyroids there were many minute follicles lined by low-cuboidal epithelium, and thick septa of connective tissue infiltrated with lymphocytes traversed the organ. The significance of this lesion is difficult to assess. Four of 6 patients showing this change were stated to have been beyond 1500 yds.

*Pituitary Body.* Six pituitary glands were available in histologic sections. In K-50 the basophilic cells were remarkable for their large

size. A few were vacuolated. They were present in groups in all parts of the gland. The eosinophilic cells, on the contrary, were minute and inconspicuous. The changes in the pituitary body were much less striking than in K-42, Figure 37. The testis of K-50 is illustrated in Figure 105.

TABLE XLIV  
*Group III: Thyroid Gland*

Observations	Hiroshima				Nagasaki			
	311	Sub-group		322	311	Sub-group		322
		312	321			312	321	
Gross specimens available	8	1	4	2	5	3	1	6
Histologic specimens available	4	0	0	1	4	2	1	5
Slight increase of interstitial tissue, and lymphocytic infiltration	1			2	1			2

TABLE XLV  
*Group III: Mouth and Neck Organs*

Observations	Hiroshima				Nagasaki			
	311	Sub-group		322	311	Sub-group		322
		312	321			312	321	
Histologic specimens available	7	1	4	2	6	3	1	6
Gingivitis, all types	3				1			1
Hemorrhagic	2							
Necrotizing	1				1			
Necrotizing focal tonsillitis	4	1	2	1				
Scars of tonsils	1							
Chronic glossitis								1
Laryngitis					1			

One other pituitary body showed slight vacuolation of the basophils, whose number, however, was small. The patient was a 78-year-old woman. This change was found constantly in senile females.<sup>77</sup>

*Mouth and Neck Organs (Table XLV).* Necrosis of the tonsils sometimes occurred in this group, but it usually was focal rather than diffuse. In one case microscopic sections showed evidence of necrosis without leukocytic infiltration, but there was hypoplasia of the bone marrow. In others, in which the marrow was not aplastic, polymorphonuclear cells were found within the necrotic tissue lining the crypts. There was no hemorrhage. Necrotizing gingivitis occasionally was still in evidence. There usually was no necrosis of the larynx or epiglottis.

*Skin (Table XLVI).* Petechiae in the late cases were unusual. Hair follicles often showed evidence of regeneration. There were renewed differentiation of the internal root sheath, regrowth of the papilla, decrease in the thickness of the basement membrane, and a new hair shaft was found on its way outwards through the plug of keratinized epithelium at the mouth of the follicle. A completely atrophic follicle showing all of these changes described in group II is illustrated in Figure 141. In

TABLE XLVI  
Group III: Skin

Observations	Hiroshima				Nagasaki			
	Sub-group				Sub-group			
	311	312	321	322	311	312	321	322
Gross specimens available	9	1	4	3	6	3	1	6
Epilation	5		1	1	2	1		3
Hemorrhages					3	2		1
Decubital ulcers		1		1	3	2		2
Eczema			1					

Figure 142 are shown the beginnings of the regenerative process. This process of atrophy and regeneration was a recapitulation of the usual processes of loss and replacement of the hair. It has been described in detail by Auburtin<sup>6</sup> (Fig. 143). In the adult, hair usually grows from the same root for 3 or 4 years. Then there is failure of differentiation of the internal root sheath, the basement membrane thickens, pigment becomes irregularly distributed, and the hair is extruded, just as in the irradiated persons. Regeneration takes place apparently from the same follicle and the keratinized tip of the new hair, capped with a new internal root sheath, burrows its way through the old plug at the mouth of the follicle. In the irradiated person the process was condensed in time and involved the great majority of follicles. Perhaps also in these follicles the atrophy was more complete, the thickening of the glassy membrane was greater, but regeneration nevertheless occurred. Regeneration was evident clinically in individuals with severe epilation within 7 weeks after the explosion.

In emaciated individuals of this group decubital ulcers occasionally occurred, as was to be expected.

## DISCUSSION

### *Mechanism of Death*

The factors responsible for death from radiation effect are not entirely clear, especially in those patients dying within the first 2 weeks. It is possible experimentally with x-rays to produce deaths "under the beam" if the dose is delivered at a sufficiently rapid rate.<sup>39,69,83</sup> Henshaw<sup>39</sup> has demonstrated histologically the widespread damage to tissue that may occur within 3 hours in animals receiving 25,000 to 50,000 r. at a rate of 250 r. per minute. Whether any of the population at Hiroshima and Nagasaki undamaged by burns or trauma died of such direct destruction of tissue within the first few hours is not known, since the first autopsies were not performed until 3 days after the bombing. During these first few days no clinical records of blood pressure were made, nor of fluid

balance studies. Thus the contribution of "shock" to radiation sickness cannot be evaluated.<sup>60</sup>

There is evidence of widespread damage to tissue of the same histologic pattern as that seen in animals succumbing within the first day after overwhelming doses of radiation. In the absence of bacteriologic studies, however, it is not possible to state with certainty that bacteria did not contribute to the early mortality, for even minor injuries and burns did not often escape infection. At 11 days the intestine of a patient at Nagasaki already showed large masses of bacteria lying within necrotic and edematous tissue in the mucosa (Fig. 83).

Few experimental studies have been concerned with the study of the generalization of infection after irradiation. Warren and Whipple<sup>93</sup> did not find evidence of an overwhelming bacterial invasion from the intestines of dogs dying within the first 4 days. Chrom,<sup>16</sup> using heavy doses of x-rays, found bacteria localized to the mesenteric lymph nodes within the first few days and generalization only after the seventh day. Similarly, after neutron or x-ray irradiation of animals, Lawrence and Tennant<sup>49</sup> found the blood and tissue cultures to be sterile within the first 4 days of radiation, but that "as the doses are decreased and the animals live longer, bacteremia is a usual finding and infection probably a more important factor in the cause of death."

After the second week heavily infected necrotic and ulcerated lesions in the skin, mucous membranes, and respiratory tract were invariably observed, and there was also in some instances, although cultures were not made, morphologic evidence of generalization of the infection with masses of bacteria in freshly fixed organs as remote from the surface as the brain (Fig. 114), bone marrow (Figs. 66 to 68), and eye.<sup>95</sup> Thus, in those patients who survived longer, infection appears to have become an increasingly important factor. Even localized infections can, under certain circumstances, contribute to the depression of bone marrow with resultant leukopenia and anemia.<sup>19</sup> Many of the lesions are indistinguishable from those seen in patients with aplastic anemia of other causation where, again, bacterial infection likewise is of primary importance in the mechanism of death. Infection, once established, can also affect the regenerative process in the tissues.

The definitive answer concerning the rôle of bacteria in increasing mortality and preventing recovery following irradiation will not be forthcoming until aseptically reared animals are exposed.

The interpretation of the histologic changes observed in these patients is rendered difficult not only by the factor of infection, but by the possible



influence of one damaged organ upon another. As far as hemopoiesis is concerned, Lawrence, Valentine, and Dowdy,<sup>50</sup> in cross-circulation experiments, found no evidence of an immediately operative humoral effect. Experiments of this type, however, do not completely solve the problem. An example is the appearance of "castration cells" in the pituitary body after destruction of the spermatogenic tissue of the testis. The careful analysis necessary before ascribing any changes to the direct action of ionizing radiations is well illustrated by the series of studies of the adrenal of the rabbit carried out by Engelstad and Torgersen.<sup>23,25,81</sup> In the last of a series of papers, Torgersen<sup>81</sup> concluded that the changes originally ascribed to the irradiation of the adrenal were in fact brought about indirectly. The histologic changes themselves that are observed after radiation are not specific, and every change can individually be produced by some other means.

### *Mitosis and Cell Activity*

Some of the effects of ionizing radiation on mitosis and activity of cells are now well known. The first autopsies came too late to detect whatever effect there may have been upon suppression of mitosis, which is stated by some to be a sensitive indicator of the action of ionizing radiations.<sup>87</sup> During the first few days many of the mitotic figures in the intestine, spleen, and elsewhere were atypical, and numerous bizarre cells, some with giant nuclei, appeared in these tissues. In the hemopoietic tissues the cells were so atypical as to resemble those found in Hodgkin's disease. "Micronuclei" appeared as in cells under the influence of colchicine.<sup>14,80</sup> Similar effects on mitosis and the presence of atypical cells have frequently been noted in the heavily radiated tissues of animals and men.<sup>26,57</sup> It is probable that most of these cells are not long viable, since after the second week they became increasingly rare in the tissues of the atomic bomb patients. Maximow<sup>57</sup> came to the same tentative conclusions in his studies of irradiated connective tissue, although he did not consider them definitive. The proliferative capacity of many tissues, however, if at all damaged, was soon restored, as demonstrated by the reticulum cells in the hemopoietic tissues, and the Sertoli cells in the testes. Phagocytic activity likewise was in evidence in the ingestion of red blood cells by the reticulo-endothelial cells of the bone marrow and spleen. Subsequently hemosiderosis developed, as described in the early observations of Heineke.<sup>34</sup> Again the factor of infection must be taken into account in considering the pathogenesis of this process.

*The Blood-Forming Organs*

The sequence of changes in the peripheral blood in the population exposed to the atomic bomb was, in general, similar to that known to occur in animals and men exposed to adequate rapidly administered doses of ionizing radiations, as contrasted with repeated minute doses.<sup>44,53</sup> The first counts were made too late, however, to observe the initial leukocytosis so commonly found in the first few hours<sup>21,37,38,50</sup> in irradiated animals and patients. The initial polymorphonuclear (and with relatively small doses, lymphocytic<sup>37</sup>) leukocytosis probably represents a "mobilization rather than new formation"<sup>11</sup> of cells.

The first counts in the patients, made at Nagasaki on the first day after the bombing and at Hiroshima on the fourth, already indicated a leukopenia which became increasingly severe during the succeeding weeks.<sup>47</sup> The platelet counts fell later than the leukocyte counts and the erythrocyte levels sank more gradually than either.<sup>21,60</sup> Despite the very severe aplastic anemia, young forms of the white and red blood cells sometimes appeared in the peripheral blood during the first 10 days.

The disappearance of mature lymphoid cells from the lymph nodes, spleen, and thymus was striking even in the first available sections of the fourth day. Experimentally, during the administration of massive doses of x-rays, Henshaw<sup>39</sup> found striking destruction within the first 3 hours after the radiation was started. In the atomic bomb patients the cells seemed to undergo autolysis *in situ* and there was little evidence of their phagocytosis as described by Tsuzuki,<sup>83</sup> Henshaw,<sup>37</sup> and others. The slow restoration of the lymphocyte count was associated in these patients as well as in experimental animals with the slow recovery of the lymphoid tissues observed histologically.<sup>11,21</sup>

Similar rapidly destructive changes in the bone marrow were produced by Henshaw<sup>39</sup> within 3 hours during massive irradiation. In the patients, tissue from a cancellous bone was not available until the tenth day, but at that time there was almost total loss of the usual hematopoietic substance. In the subsequent 3 weeks, some marrows showed persistence of erythroblastic foci despite disappearance of granulopoietic tissue. This finding in irradiated bone marrows has been reported by Dunlap.<sup>21</sup> Bloom's<sup>10</sup> statement that the erythroblasts are exceedingly sensitive was not confirmed in the present material.

Remarkable was the resistance to destruction of the reticulum cells. A skeleton of these elements remained in the lymph nodes, skin, and bone marrow despite complete destruction of all mature cells. This

radio-resistance of the "macrophage system" has been noted by Bloom.<sup>10</sup> The reticulum cells retained their morphologic integrity even after the devastating doses employed by Henshaw.<sup>30</sup> Remarkable also was the regenerative capacity of reticulum cells. Proliferation of these elements was already in evidence by the sixth day in the earliest available specimen of a long bone. Different patients varied in the extent of regeneration, but when it occurred, the long as well as the flat bones took part in the regenerative process. The large agranular pale cells might form focally or diffusely distributed masses of stellate or rounded elements. They were described very early by Heineke<sup>30</sup> in regenerating marrows following external radiation, and by Martland<sup>55</sup> in marrows of patients who had ingested radium or mesothorium. Martland considered this "regenerative leukopenic anemia" the result of internal, as contrasted with external, radiation which in his view resulted in an aplasia of the marrow. This is certainly belied by the appearance of the marrows of many patients dying some 6 weeks after the atomic radiation. Hyperplastic bone marrows likewise may occur in individuals with "aplastic anemia" not produced by ionizing radiation, as described by Rhoads and Miller,<sup>71</sup> who also described large pale cells similar to those of the early phase of regeneration after the atomic bomb radiation. In the heavily irradiated patients these cells appeared at first to differentiate by a gradual series of transformations into plasma cells. After the first month, however, such cells appeared in some patients to acquire azurophilic granules and to be connected by a series of transition forms with myelocytes. Often deeply basophilic blast cells were present in such marrows, but in relatively small numbers, suggesting that the reticulum cells can be transformed quickly into myelocytes. Later, hyperplastic marrows of more usual structure, but retaining for some months unusually large numbers of plasma cells and lymphocytes, might be seen. This sequence of events was actually traced by repeated bone marrow aspiration studies in a series of patients<sup>47</sup> and is not described merely from the histologic appearance of bone marrows of patients dying at various times after the irradiation.

As Dunlap<sup>21</sup> and Rhoads and Miller<sup>71</sup> have pointed out, the cellularity of a bone marrow is not necessarily an index of the rate of delivery of cells to the peripheral blood. The nature of the "maturation defect" in some of the hyperplastic marrows is completely unknown. Perhaps the "defect" lies in the supply of some essential substance, as in pernicious anemia, or possibly the infections that accompany the phase of severe depression of the marrow are responsible. Certainly a major

therapeutic effort should be made to eliminate the factor of infection pending the resumption of more orderly hemopoiesis.

The mechanism of hemorrhage after irradiation recently has been illuminated by the studies of Allen and Jacobson<sup>4</sup> who demonstrated the appearance in the blood of a substance with the properties of heparin. This work, unfortunately, was unknown to the physicians in the field at Hiroshima and Nagasaki. It must be stated, however, that these observers were puzzled by the appearance of hemorrhages in patients whose levels of platelets had not fallen to values when hemorrhages usually occur.<sup>47</sup> The source of the heparin is unknown although mast cells were found early in relatively large numbers in the bone marrows and lymph nodes of the irradiated patients and later in the submucosa of the intestines beneath ulcerative lesions. The presence of mast cells in large numbers in the tissues is not necessarily indicative of hyperheparinemia, as Oliver<sup>66</sup> has shown in his observations of dogs with mast cell tumors. Toward the end of the first month, however, platelets in patients with a severe radiation effect often fell below 10,000 per cmm., a level at which cutaneous hemorrhages could be expected. The importance of thrombopenia as such in the mechanism of hemorrhage has likewise been recognized by Allen and his co-workers.<sup>3</sup>

Eosinophilia occasionally has been found in animals and patients after irradiation and was observed in some of the Nagasaki patients. The mechanism of the eosinophilia is obscure. There is some question, however, as to whether there may not have been exposure to renewed hookworm or other infections by parasites in these patients, who were thrust into unhygienic surroundings after their displacement from the city. It is interesting to note that Jacobson and Marks<sup>44</sup> found no evidence of eosinophilia in animals irradiated daily for long periods with 0.11 to 8.8 r. of gamma rays.

### *The Gonads*

It was realized from the first by Albers-Schönberg,<sup>1</sup> the discoverer of the sterilizing effects of x-rays, but often lost sight of by his successors, that mere histologic changes in the testes must be distinguished from the condition of sterility by the test of mating after an adequate interval of time. As Warren<sup>88</sup> has stated, the testis is not far different histologically whether in temporary or in permanent azoospermia; in the former condition a lesser number of germinal epithelial cells appears to have been destroyed. In the patients exposed to the atomic bomb, the testes showed profound destructive changes beginning as early as the

fourth day when the first material was available for histologic examination. The changes became more profound in succeeding weeks, but additional factors—infection and starvation—probably became concerned in the pathogenesis. In many of these patients a few spermatogonia still remained in close association with the basement membrane. A controlled clinical study was performed some 10 weeks after the explosion by examining active and well nourished men who had been relatively close to the center. In many of these, sperm counts revealed hypospermia and azospermia in contrast with the findings in a group of prisoners who had been just beyond the range of radiation. Follow-up studies on the exposed men will be necessary, however, to establish whether permanent or temporary azospermia has been produced.

The relation of the testicular atrophy to the appearance of "castration cells" in the pituitary body is of interest. Similar changes in the pituitary body have been observed in the rat after irradiation of the testes.<sup>46,86</sup> In this process the interstitial tissue of the testes remains morphologically intact or even becomes hyperplastic. This suggests that the germinal epithelium or its derivatives may have an endocrine function. Further evidence is the appearance of "castration cells" in the pituitary body after ligation of the ductuli efferentes.<sup>85</sup>

The relative sensitivity to irradiation of the human ovary and testis has not as yet been finally established. No additional conclusive information was adduced on examination of tissues of patients exposed at Hiroshima and Nagasaki. The histologic changes were less spectacular in the ovary. The incidence of amenorrhea in the women was found to be inversely proportional to their distance from the center, but data of this type are less objective than sperm counts in the men and are somewhat obscured by the high incidence of "war amenorrhea"<sup>47</sup> in Japan, and perhaps by uterine bleeding of other than menstrual origin such as may accompany purpura. According to Dunlap,<sup>22</sup> the sterilizing dose for men is probably less than that for women. Under the conditions of irradiation described by Peck and his associates,<sup>67</sup> it was possible to produce permanent amenorrhea in 50 per cent of women following the administration to the tissues of 125 to 150 r. Above 625 r., almost all female patients were permanently castrated. It seems possible that non-fatal doses of radiation to the body as a whole may produce permanent sterility in some instances. On the other hand, even massive doses to the ovary may not result in permanent amenorrhea.<sup>45</sup> It is interesting to note, moreover, that conception can take place in some patients despite post-irradiation amenorrhea.<sup>58</sup> Species differences may exist in

the relative sensitivity of the gonads of the two sexes. Thus, in the mouse, Lorenz and his associates<sup>53</sup> found that upon protracted irradiation with small doses the injurious effect was cumulative and irreversible for the ovary but not for the testis.

### *The Fetus and the Germ Plasm*

It is now well known from clinical experience that irradiation may exert damaging effects on the unborn child without producing abortion. The resistance of the corpus luteum is well known.<sup>73</sup> After the application of a 60 per cent skin erythema dose (600 r. by his technic) to the tissue of the ovary, Mayer and his co-workers<sup>58</sup> found that abortion could be induced in 96 per cent of pregnant women. If an abortion does not result after large doses of radiation to the pelvis, the incidence of microcephaly and idiocy in a child is high, especially if the irradiation is administered before the end of the fourth month of pregnancy.<sup>22</sup> No statistics are available concerning such occurrences among the Japanese after the atomic bombings. The disrupted municipal facilities prevented accurate collection of vital statistics. It is probable that infant mortality was high because of the appalling hygienic conditions that prevailed after the atomic bombings and the great typhoons that followed.

Non-sterilizing irradiation of the female pelvis, when carried out before conception, is stated not to be followed by an unduly high incidence of abnormal offspring.<sup>22,30,63,64</sup> The studies concerning fertility made to date by questioning radiologists<sup>42</sup> or technicians<sup>65</sup> are inconclusive, since large masses of data are required together with adequate control material.

Effects on the germ plasm, as has been pointed out by the pioneer studies of Muller<sup>61,62</sup> on fruit flies, and by Snell<sup>78</sup> of Muller's laboratory on mice, must be studied with care over a series of generations before conclusive evidence can be forthcoming. These changes in the germ plasm are entirely distinct from those that can be produced in the embryo by radiating the products of conception. Muller<sup>61</sup> and others have demonstrated changes of two types in the germ plasm itself consequent upon irradiation: (1) gene mutations, by which is implied a change in the actual composition of the genes, and (2) chromosome transformation, whereby is implied a change in the position of the genes within the chromosomes. Such gene mutations as may result are usually recessive, and can be detected no sooner than in the third generation, and then only by inbreeding. Such mutations are more apt to be manifested by the elusive effects of lower "general vitality or efficiency than conspicuous

morphological abnormalities.”<sup>62</sup> The most common situation, moreover, is for the gene or chromosome mutations to be lethal to the embryo. These fundamental facts were established for *Drosophila* by Muller and have been confirmed by others for many species. In mammals, Snell,<sup>78</sup> working with mice, found evidence of translocation changes in the chromosomes manifested by heritable lowered fertility (“semi-sterility”). Small litters developed in stocks the male ancestors of which had had their testes exposed to doses of between 600 and 800 r. Such heritable changes can be produced by irradiating mature spermatozoa as well as the spermatogonia, although a larger dose is needed to effect them in the latter.<sup>62</sup> All of these changes followed acute irradiation. Lorenz and his associates<sup>53</sup> could find no evidence of an effect on the germ plasm when minute doses of gamma rays were applied at low intensity over long periods to successive generations. The special sensitivity of the spermatozoa has stimulated Muller<sup>61</sup> to say that “it should, therefore, be mandatory for a man to abstain from acts of reproduction for some 2 months after his testes have been exposed to any considerable dose of radiation.” Likewise the genetic consequences of mass irradiation on large populations have been considered recently by Muller<sup>61</sup> who has stated “if, as in flies, a total dose of 50 r. units, applied to the spermatozoa, results in a mutation frequency about equal to the natural mutation frequency, but added to it, and if this frequency is taken as being about one mutation in 10 to 20 germ cells, then I should hold the effect important.” Thus, as the use of atomic energy becomes more and more widespread, the necessity of protecting populations becomes increasingly imperative, even considering the quantitative factors that have been discussed in the preceding paragraphs.

### *Neoplasia*

The existence of a very high incidence of deep burns involving large areas of skin would suggest that carcinoma arising in the epithelium covering the scars will be observed. It will be of interest to determine whether patients who had flash burns, and who consequently were exposed to intense ultraviolet radiation, will have a higher incidence than those whose burns were produced merely by contact with flame or hot objects. Whether the additional factor of exposure to gamma radiation will increase the incidence of carcinoma arising in the burned integument likewise remains to be determined.

Radiation dermatitis, whose precancerous nature has been described by Wolbach,<sup>90</sup> Saunders and Montgomery,<sup>76</sup> and others, was not found

among the survivors. An erythema dose to the skin as a whole would be fatal, especially if the soft rays responsible for the latter were mixed with more penetrating radiations.

Bone sarcomas in man have been described after radiation of benign tumors or even normal bone.<sup>15</sup> Large doses locally applied, however, appear to be required before tumors will result. Such doses again would be fatal if applied to the body as a whole. A much more palpable danger would exist from the ingestion or inhalation of radioactive material, as Martland<sup>55</sup> showed in the radium dial painters. Lisco, Finkel, and Brues<sup>51</sup> already have produced bone tumors experimentally with plutonium and radioactive fission products. In the patients subjected to the atomic bomb there was no evidence of the introduction of radioactive material.

Ovarian tumors have been produced in mice with remarkably small doses of gamma rays—as little as 50 r. given at one time, or 0.11 r. given daily for a total of 90 r.<sup>44,53</sup> The tumors—granulosa cell, tubular adenoma, or luteoma—appear after atrophy of the germ cells. Nothing comparable has as yet been observed in human pathology, but women exposed in the bombed cities should certainly be studied with care over a long period.

The evidence concerning the leukemogenic action of x-rays has been reviewed by Furth.<sup>28</sup> Chronic irradiation has repeatedly been proved to increase the incidence of leukemia in susceptible strains of mice.<sup>40,53</sup> Also, radiologists are known to have a death rate from leukemia greater than that of other physicians.<sup>54</sup> Relatively little is known about the effect of single doses of penetrating gamma rays. Furth concluded that x-rays are only weakly leukemogenic and then only after a long incubation period. There has not been an unduly high incidence of leukemia at Hiroshima and Nagasaki to date as far as can be determined,<sup>91</sup> and the single case of monocytic leukemia is considered purely adventitious. However, further observation will be necessary before this question is decided.

Little material is at hand by which any possible effect on the growing tissue of bone can be evaluated. In clinical experience, radiation possessing certain qualities and locally applied has been found to produce damage to the epiphysis without visible injury to the skin. Whether radiation of the intensity necessary to produce this effect is within the tolerance range when applied to the whole body remains to be determined by biometric and roentgenographic studies of surviving irradiated children of the Japanese cities.



## SUMMARY AND CONCLUSIONS

The explosion of the atomic bomb produced mechanical, thermal, and ionizing radiation injuries. The last were quantitatively the least important in the production of casualties.

Direct blast injuries analogous to those inflicted by high explosives were almost unknown among survivors, as indicated by an incidence of ruptured eardrums of about 1 per cent. Almost universal, however, was damage produced by flying glass and the falling beams of wooden houses. The more severe injuries were rare since those that had been severely hurt were killed by fires that swept the city before rescue operations could be instituted.

The burns among survivors were largely of the "flash" type, the result of an exceedingly large quantity of radiant heat acting for an exceedingly brief interval. The effects of exposure extended to approximately 4000 yds. from the bomb. Only survivors in the direct path of the rectilinear rays were involved, so that the burns were of a sharply outlined "profile" or "mask" type. Depigmentation at the center with marginal hyperpigmentation of the burns was prominent in patients close to the bomb, but at greater distances the entire exposed surface became intensely pigmented, and the pigment showed no tendency to fade within 4 months. There was histologic evidence that depigmentation occurred without destruction of the squamous epithelium of the surface, suggesting the action of specific wavelengths.

Even minor injuries and burns became serious foci of infection in persons who also suffered the leukopenia resulting from radiation.

The effects of ionizing radiations resembled closely those produced by total body x-irradiation of animals and men. A special effort was made to center the study of the lesions in patients who had sustained little or no other injury. The effects of ionizing radiations were observed in varying degrees of severity in poorly shielded patients who had been within approximately a mile from the bomb.

Nausea and vomiting occurred in many adequately exposed persons within a few hours after the bomb. The earliest autopsy material was from persons dying "mysteriously" with symptoms of severe diarrhea and fever on the third day after the bomb. In them, epilation and purpura had not had time to appear. After the end of the second week, however, these signs became manifest, and the infective complications of an aplastic anemia became increasingly prominent. Leukopenia had been observed within the first few days after the bombing. After the sixth week, the bone marrow tended to recover and the mortality de-

clined sharply, although an occasional patient succumbed to organizing pneumonitis or ulcerative enteritis. At necropsy, changes were found in the skin, gastro-intestinal tract, gonads, and hemopoietic tissues.

### *The Skin*

In a few cases there were suggestive epithelial changes at the margins of ulcerative lesions in patients dying in the third week, but since most persons who received more than an erythema dose over the whole body died during the first confused days when autopsies were extremely rare, little material was available for study. Epilation in both men and women usually began 14 to 20 days after the bomb. It involved chiefly the scalp in a distribution resembling that of ordinary baldness. Histologically, the mechanism appeared to be entirely analogous to that of the usual processes of loss and replacement of the hair, arrest of mitosis in the matrix, failure of differentiation of the internal root sheath with extrusion of old hair, and finally (some 2 months after irradiation) renewed differentiation of the internal root sheath with penetration of the new hair through the old external sheath to the surface.

### *Gastro-intestinal Tract*

Typical radiation changes were seen in the intestines of persons dying as early as the fourth day. These consisted of the appearance of bizarre cells, some with enormous nuclei possessing a coarse chromatin network and a large body of cytoplasm. Some cells were found in atypical mitosis and tripolar mitotic figures were observed. In one patient who died on the tenth day, the cytoplasm and nuclei of the squamous epithelial cells of the pharynx and tongue were remarkably swollen, and fragmentation of the nuclei was observed.

### *Gonads*

Even at the fourth day remarkable changes were found in the testes, with detachment of the germinal epithelium together with an increase in Sertoli cells. Toward the end of the first month, there was almost complete loss of germinal epithelium. After the fifth week the tubules began to display thickening of the basement membrane and there were hyaline deposits restricting the lumina of the interstitial blood vessels. There was questionable hyperplasia of the interstitial tissue after the end of the sixth week. Clinically, there was a remarkable decrease in the count of spermatozoa of patients who had been close to the bomb. How permanent this will be is at present unknown. "Castration cells" were found occasionally in the pituitary body.

Much less striking changes were observed in the ovary. A few primary follicles were in process of atresia. The most usual finding was that of the absence of developing follicles despite the persistence of primary follicles. The endometrium showed an absence of corpus luteum effect.

### *Lymphoid Tissues*

When first seen, after 3 days, there was a remarkable degree of atrophy of the lymphoid tissue, including those of the spleen, leaving nothing but the reticular skeleton. Beginning on the fifth day, however, large numbers of atypical mononuclear cells resembling lymphoblasts or Reed-Sternberg cells began to appear. These gradually decreased in number during the following 3 months and in a few instances secondary follicles had reappeared by the end of this time both in the spleen and lymph nodes.

### *Bone Marrow*

Even within the first week, in heavily irradiated individuals, almost all mature myeloid and erythroid tissue had disappeared, but there already was evidence of proliferative activity on the part of the reticulum cells. During the first month such proliferative activity became remarkable in many cases, but the products were largely atypical reticulum cells and plasma cells. In some patients, after various lengths of time, there was renewed differentiation into granulopoietic and erythropoietic tissue, and in some, who died toward the end of the sixth week, actual hyperplasia of this tissue was observed, although peripheral leukopenia had been noted.

After the sixth week more and more bone marrows tended to show hyperplasia of myeloid cells and the incidence of leukopenia decreased. In all of these marrows, however, considerable numbers of reticulum cells, plasma cells, and lymphocytes persisted in increased numbers. As the marrow recovered, polymorphonuclear leukocytes became numerous in the lesions and hemorrhage ceased to be an important factor. The important lesions at necropsy at this time were either a necrotizing pneumonia, sometimes in process of organization, or an ulcerative enteritis. Thrombocytopenia was at its lowest level at about the third to fourth week. Thereafter there was rapid recovery. Anemia also was severe during the third to fifth weeks in many patients, but in others it gradually reached its lowest levels thereafter. Recovery in these people was sluggish. This has been associated with dietary deficiency as well as with the effects of the ionizing radiation and of the infections that followed.

No defense has as yet been developed against the destructive effects upon cells subjected to massive amounts of ionizing radiations. Hope

for success in treatment lies in the remarkable resistance of the reticulum cell and the tendency of the marrow ultimately to recover. Pending the resurrection of the marrow, the main therapeutic problems are those of hemorrhage and infection. Despite recent advances, new and more efficient methods for meeting this double challenge remain to be discovered.

Among the many problems that are still to be investigated among the populations of Hiroshima and Nagasaki are: (1) Whether permanent damage of such nature as to reduce the life span was inflicted upon survivors who have apparently recovered. (2) Whether there will be an increased incidence of neoplasia (including leukemia) among the burned or irradiated patients. (3) Whether the growth of irradiated children has been impaired. (4) Whether permanent sterility was induced in any group of survivors. (5) Whether genetic changes will appear, as indicated by decreased fertility, or demonstrable anatomic or physiologic changes. These problems will require many years, or even generations for their solution, if they can be solved at all.

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### DESCRIPTION OF PLATES

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#### PLATE 121

FIG. 1. Multiple injuries by flying glass. Patient was in a standing position, approximately 5 ft. from a window, indoors in a military barracks. Upper torso was nude but he was wearing trousers, which were not penetrated by the glass. Keloidal overgrowth of connective tissue. White blood cell count was 13,000 on the 44th day. Takatomi. Male, age unknown. Approximately 1000 yds. Died on the 57th day. Army Institute of Pathology negative no. HP 151A. Bunka-Sha photograph.

FIG. 2. "Flash burns." The darker portions of a striped pattern of cloth that the patient was wearing absorbed more heat and produced the gridiron burns of the skin. The arm below the sleeve line and the unprotected face were most severely burned. The burns are very sharply outlined. Ushio. Female, age unknown. Distance unknown. Late August, 1945, approximately 3 weeks after bombing. A.I.P. neg. HP 138-d. Photo made by Japanese medical officers of Tokyo 1st Military Hospital.

FIG. 3. "Flash burns" of third degree. No protection by the hair. Partial protection of the lower extremities by cloth trousers, and complete protection of skin of abdomen by the multiple layers of a cummerbund. Burns of the back, where there was no clothing, are sharply outlined. Name unknown. Male, age unknown. Distance unknown. A.I.P. neg. HP 142. Photo made by Japanese medical officers of Tokyo 1st Military Hospital.

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PLATE 122

FIG. 4. "Flash burns." Depigmentation sharply outlined by pigmented tissue in a very narrow band. The line of the burn extends upwards on the chin. The darker material in the peri-aural region is not pigmented tissue, but is a crusted exudate and keratin. Kosugi. Female, 17 years of age. Approximately 1700 yds. 100th day. A.I.P. neg. HP 117 (K).

FIG. 5. "Flash burns." Deep chocolate-brown pigmentation. Very sharp outlines. Protection of upper portion of neck by shadow of mandible, and of nasolabial and lateral nasal grooves by the alae and nose. Enami. Male, 30 years of age. Approximately 2300 yds. 99th day. A.I.P. neg. HP 112b (K).

FIG. 6. Flash burns of skin. General view. Zone of greatest destruction of epithelium at the right. Depigmented tissue with relatively well preserved epithelium in central zone. Hyperpigmented tissue at left (see Figs. 11 and 12). K-4. Kume. Male, 32 years of age. Approximately 1000 yds. Died on the sixth day. A.I.P. neg. HM 134 (K).  $\times 15$ .

FIG. 7. Keloids following flash burns. Protective effect of shoulder straps of slip and of sleeve seam. Pigmentation at margins of the burns. Akamatsu. Female, 21 years of age. Approximately 1400 yds. Approximately 2½ months. A.I.P. neg. HP 111-b (K).

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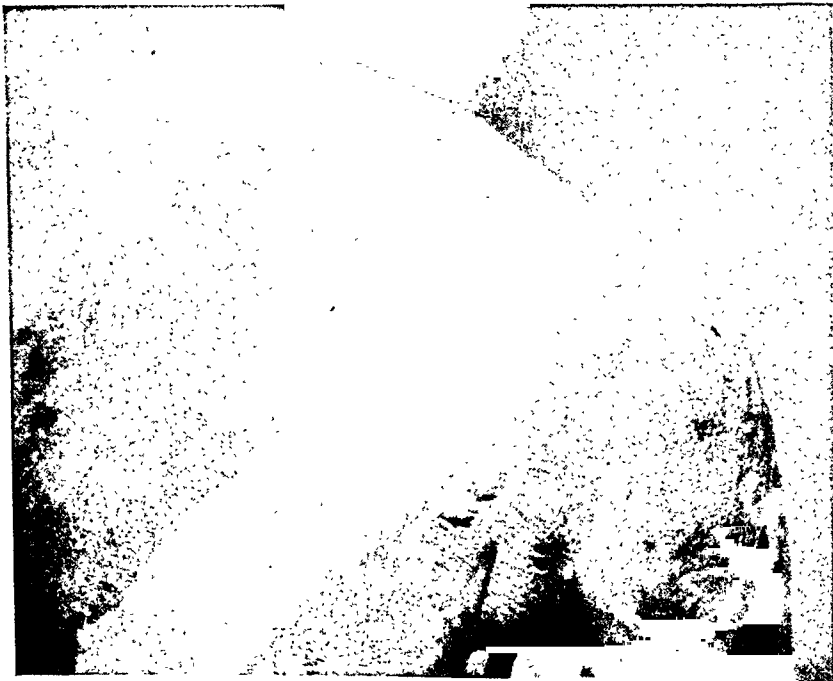


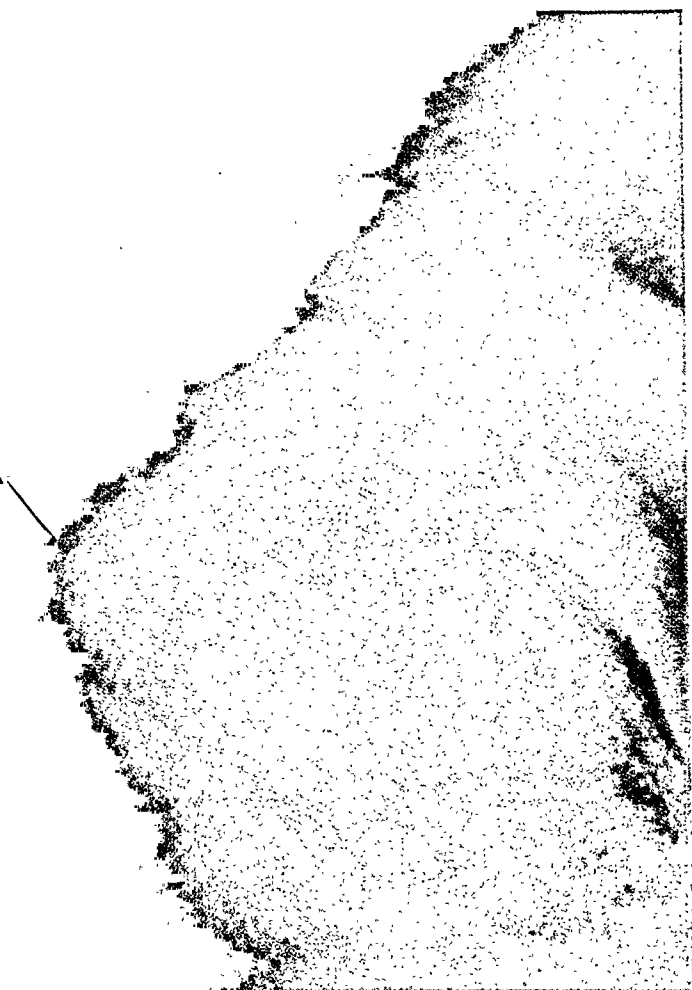
PLATE 123

FIG. 8. "Flash burns" of shoulder. Mottled depigmentation of central portions where burn was most severe, outlined by a band of hyperpigmented tissue. There is a narrow zone of depigmentation between the normal and burned skin. Maeda. Male, 40 years of age. Approximately 2300 yds. 99th day. A.I.P. neg. HP 115-a (K).

FIG. 9. Epilation of scalp. Scattered long hairs of the original growth remain. Patient was inside a wooden building at Nagasaki at the time of the bombing. Epilation began on the 19th day, 3 days after the appearance of purpura. Leukopenia persisted for 2 months, but the patient recovered. 63rd day. A.I.P. neg. NP 159.

FIG. 10. Group II. Petechiae of epicardium. K-28. Kawaura. Male, 23 years of age. Approximately 1000 yds. Died on the 26th day. A.I.P. neg. HS 307 (K).

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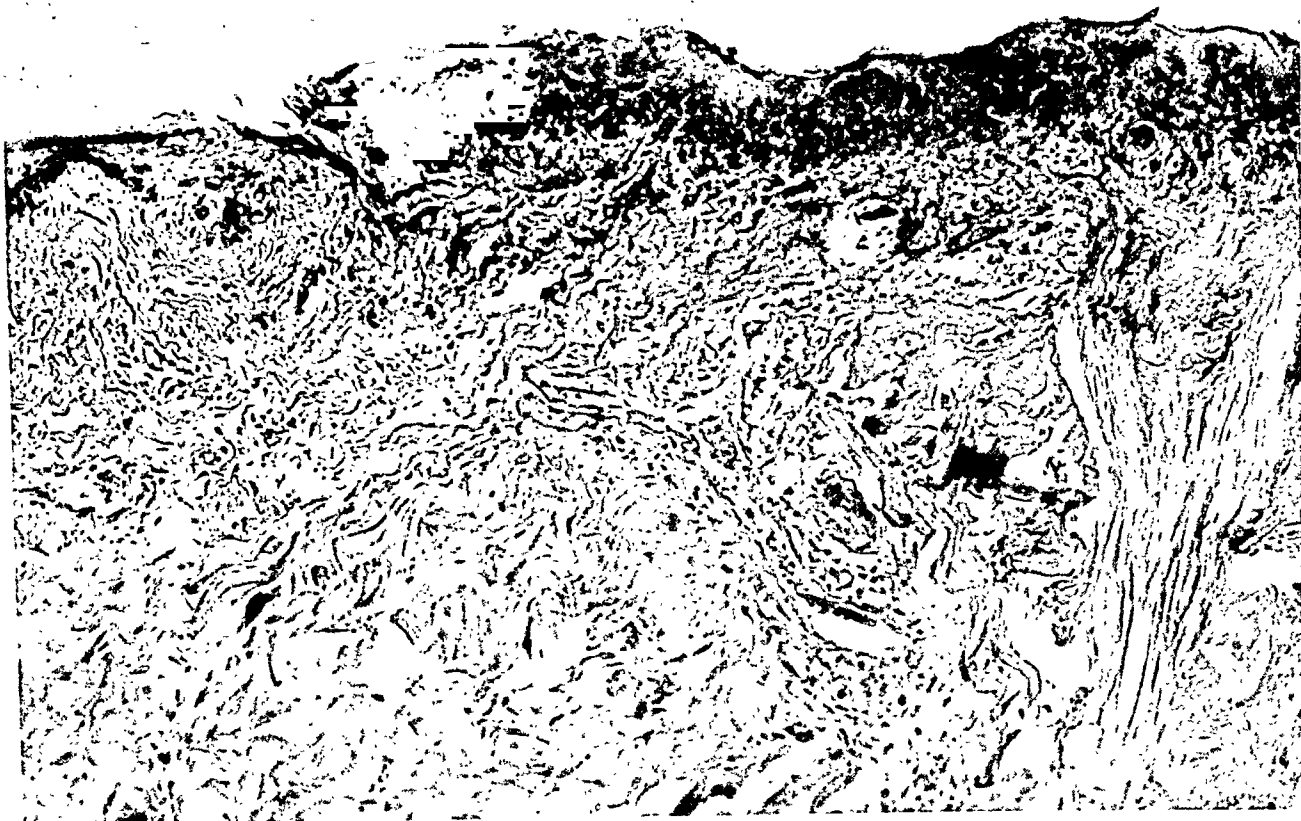


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PLATE 124

FIG. 11. Group I. Enlargement of right-hand portion of Figure 6. At right, necrosis of epithelium; infiltration with large mononuclear elements, which also are in process of necrosis. At left, well preserved epithelium without pigment. Dermal melanophores also are not seen here. A.I.P. neg. HM 133.  $\times 100$ .

FIG. 12. Group I. Enlargement of left-hand margin of Figure 6. Hyperpigmentation of skin. Large numbers of stellate melanophores among the epithelial cells. A.I.P. neg. HM 132.  $\times 200$ .



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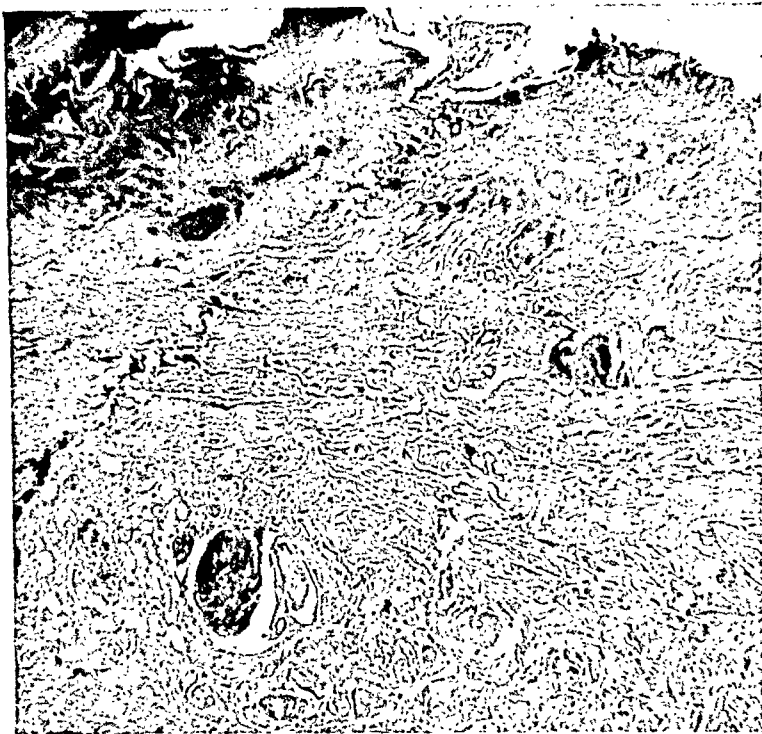
Pathology of Atomic Bomb Casualties



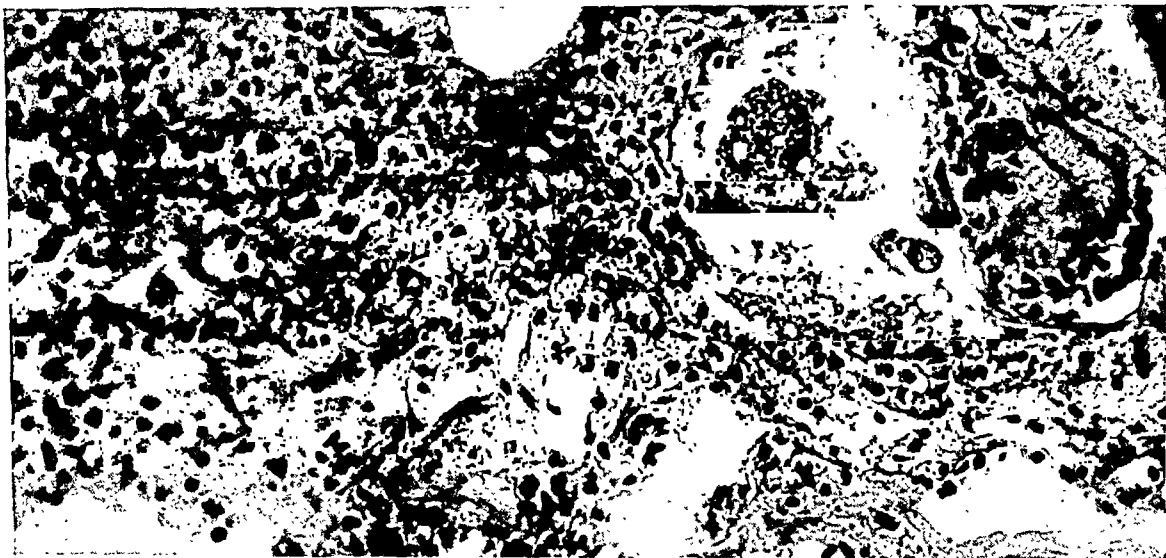
PLATE 125

- FIG. 13. Group I. Skin from burned area. Necrosis of epithelium and outer layers of derma. Bacterial masses in derma without cellular reaction. Vacuolation and shrinkage of acinar epithelium of sweat glands. Squamous metaplasia of duct of sweat gland. Bacterial mass within arrector pili muscle. K-5. Yano. Male, 39 years of age. Approximately 1000 yds. Died on the sixth day. A.I.P. neg. HM 130.  $\times 50$ .
- FIG. 14. Group I. Skin from burned area. Minute thrombus in blood vessel where wall has undergone necrosis. The exudate is almost exclusively of large mononuclear cells. K-3. Sakuma. Male, 15 years of age. Approximately 1000 yds. Died on the fifth day. A.I.P. neg. HM 113.  $\times 350$ .
- FIG. 15. Group I. Subcutaneous tissue deep to burn. Edema and myxomatous change of connective tissue. Proliferation and swelling of histiocytic and fibroblastic elements. Many mast cells are seen. From the same patient as Figure 14. A.I.P. neg. HM 118.  $\times 100$ .

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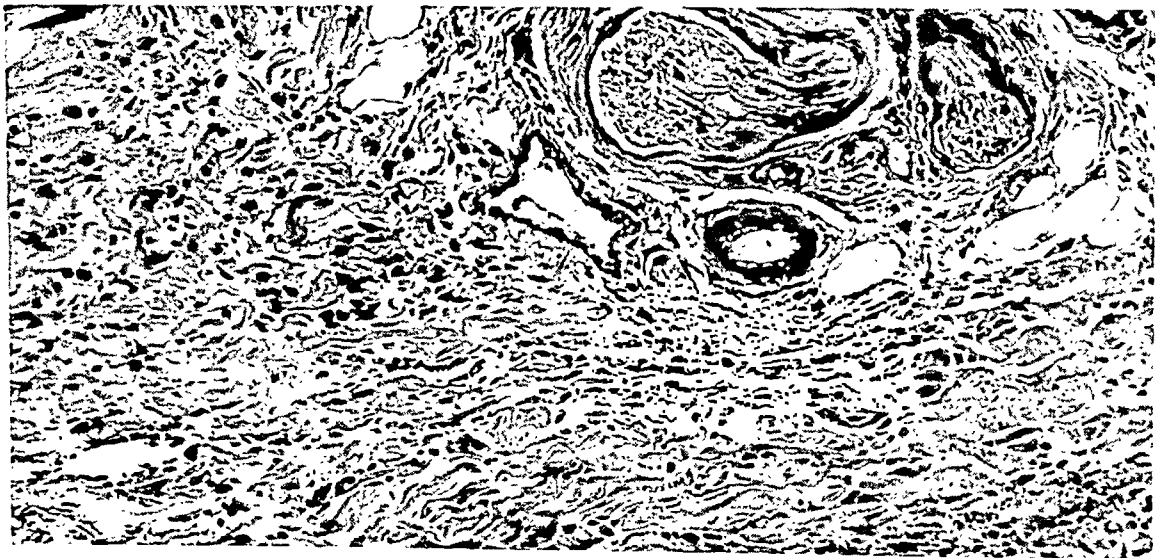


PLATE 126

- FIG. 16. Group II. Subendocardial hemorrhages of left ventricle in region of conduction bundle. K-43. Horinouchi. Male, 33 years of age. Approximately 1000 yds. Died on the 33rd day. A.I.P. neg. HS 326b.
- FIG. 17. Group II. Focal necrotizing bronchiolitis and focal necrotizing neutropenic pneumonia with surrounding hemorrhage. Relatively more necrosis than in Figure 19. K-36. Morita. Female, 21 years of age. Approximately 1000 yds. Died on the 28th day. A.I.P. neg. HS 319.
- FIG. 18. Group II. Lung. Scar of apex. Disseminated caseous tubercles surrounded by hemorrhagic parenchyma. K-40. Motoyama. Male, 29 years of age. Approximately 1000 yds. Died on the 30th day. A.I.P. neg. HS 322.
- FIG. 19. Group II. Lung. Focal necrotizing bronchiolitis and focal pneumonia with surrounding hemorrhage. The foci of necrosis are minute. K-44. Araki. Male, 22 years of age. Approximately 1000 yds. Died on the 33rd day. A.I.P. neg. HS 329.

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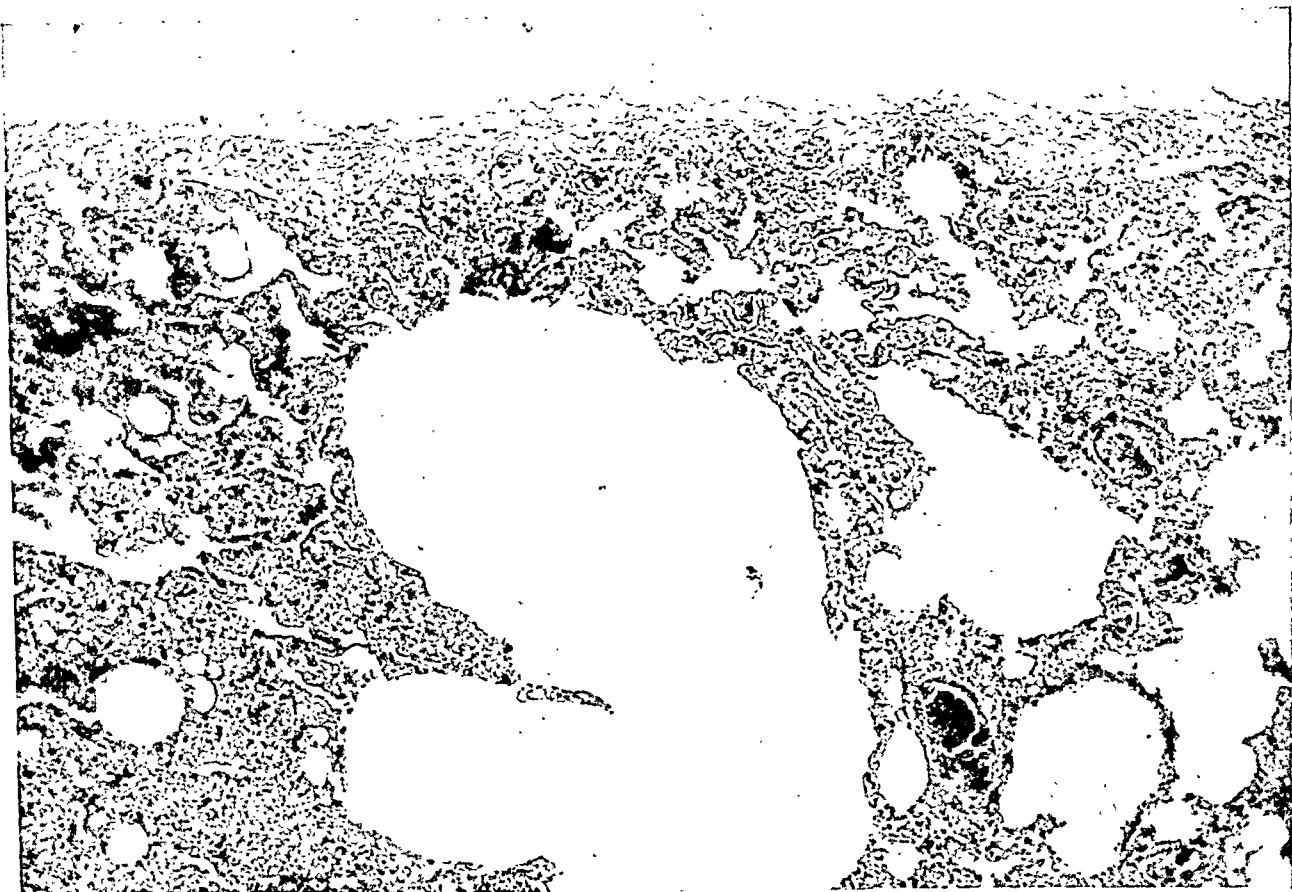
PLATE 127

FIG. 20. Group I. Lung. Focal emphysema and atelectasis. Edema of pleura. K-1. Harada. Male, 13 years of age. Approximately 1300 yds. Died on the third day. A.I.P. neg. HM 104.

FIG. 21. Group II. Heart. Perivascular and interstitial infiltration with small and large mononuclear cells and plasma cells. K-29. Murakami. Male, 22 or 24 years of age (variously stated). Approximately 1000 yds. Died on the 27th day. A.I.P. neg. HM 154.  $\times 220$ .



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PLATE 128

- FIG. 22. Group II. Lung. Necrosis of bronchiole. Bacterial masses attached to wall. Necrotizing and hemorrhagic pneumonia. K-21. Iseoka. Male, 45 years of age. Approximately 1000 yds. Died on the 24th day. A.I.P. neg. HM 145.  $\times 100$ .
- FIG. 23. Group II. Lung. Focus of "necrotizing and hemorrhagic aplastic pneumonia." Bacterial masses near center of the lesion. Lining membrane of bronchiole near right center of lesion is completely necrotic. K-47. Naka. Female, 35 years of age. Approximately 800 yds. Died on the 18th day. A.I.P. neg. HM 242.  $\times 50$ .
- FIG. 24. Group II. Lung. Edema of pleura. Necrotizing and hemorrhagic "aplastic pneumonia." K-119. Nagado. Female, 26 years of age. Approximately 1300 yds. Died on the 23rd day. A.I.P. neg. HM 288.  $\times 115$ .

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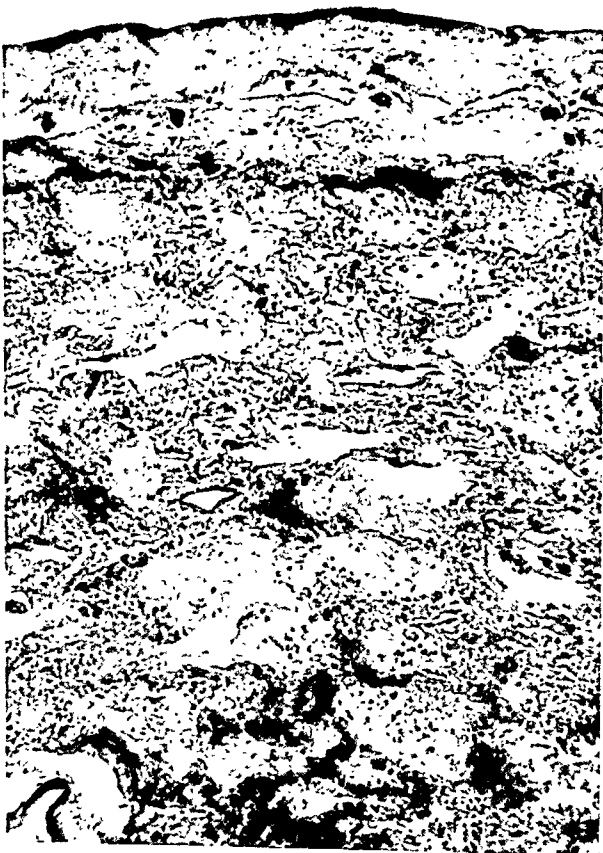




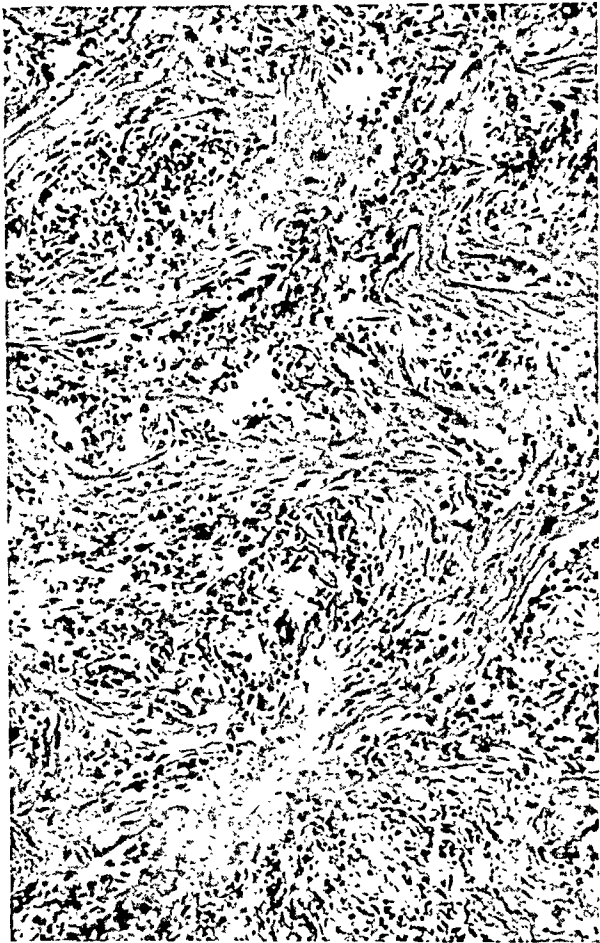
PLATE 129

- FIG. 25. Group III. Fibrous wall of abscess cavity. From the same patient as Figure 27. A.I.P. neg. HM 269.  $\times 50$ .
- FIG. 26. Group III. Lung. Organizing pneumonia. K-96. Sakoda. Male, 33 years of age. Approximately 1000 yds. Died on the 97th day. A.I.P. neg. HM 278.  $\times 130$ .
- FIG. 27. Group III. Right lung. Gangrene of upper lobe with sequestered lung tissue. Abscess cavity in lower lobe. Diffuse necrotizing and organizing pneumonia. K-50. Kijima. Male, 31 years of age. Approximately 1000 yds. Died on the 100th day. A.I.P. neg. HS 333.

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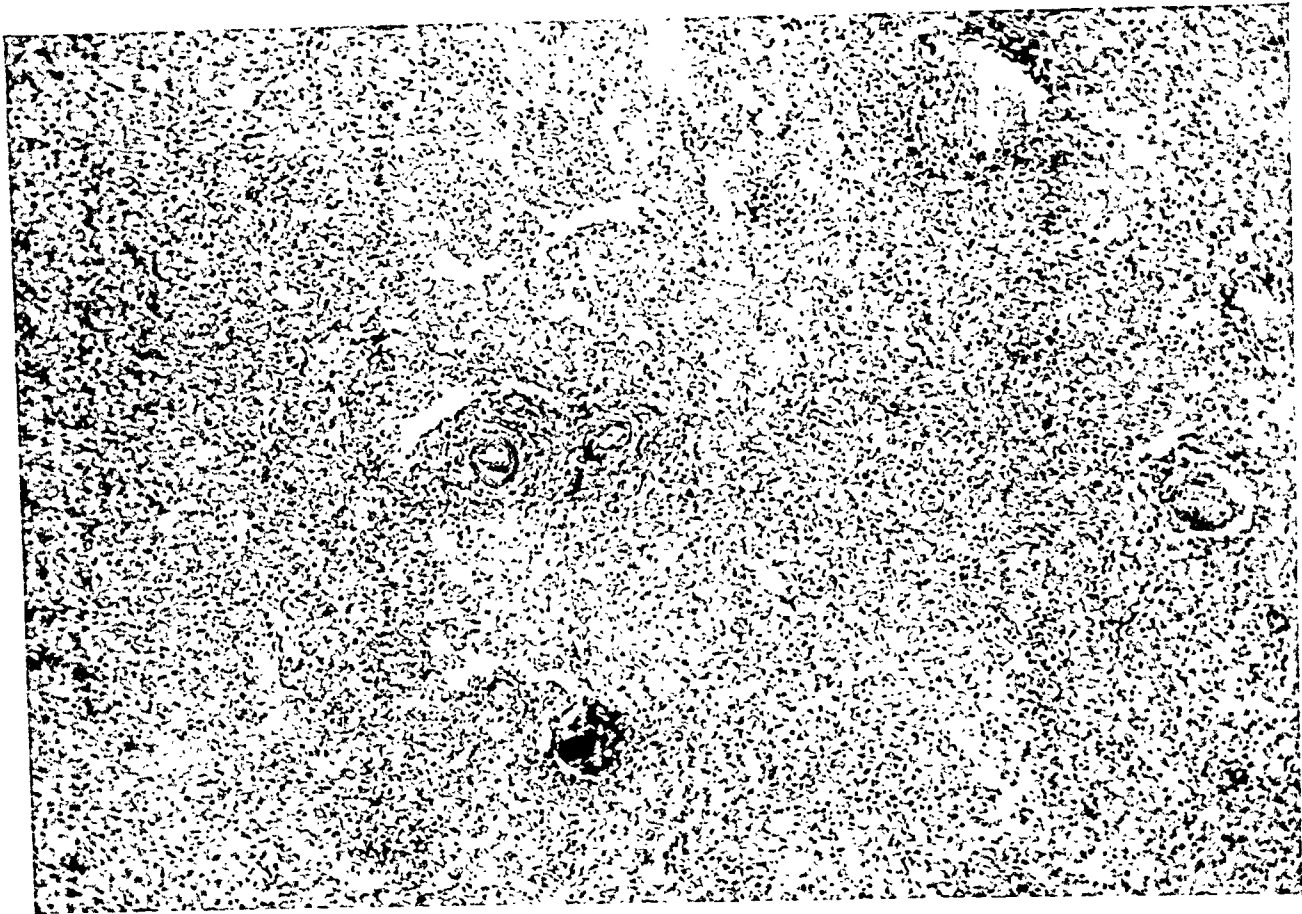
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PLATE 130

- FIG. 28. Group I. Spleen. Marked shrinkage of malpighian body. Disappearance of lymphocytes and of cells of Billroth's cords. Acidophilic refractile material in subendothelial position of central vessel. K-2. Onishi. Male, 24 years of age. Approximately 800 yds. Died on the fourth day. A.I.P. neg. HM 102.  $\times 100$ .
- FIG. 29. Group I. Spleen. Disappearance of lymphocytes from sites of malpighian corpuscles. Necrosis *in situ* of lymphocytes and of cells of germinal center. Acidophilic refractile material in subendothelial tissues of central arteries. From the same patient as Figure 28. A.I.P. neg. HM 100.  $\times 400$ .

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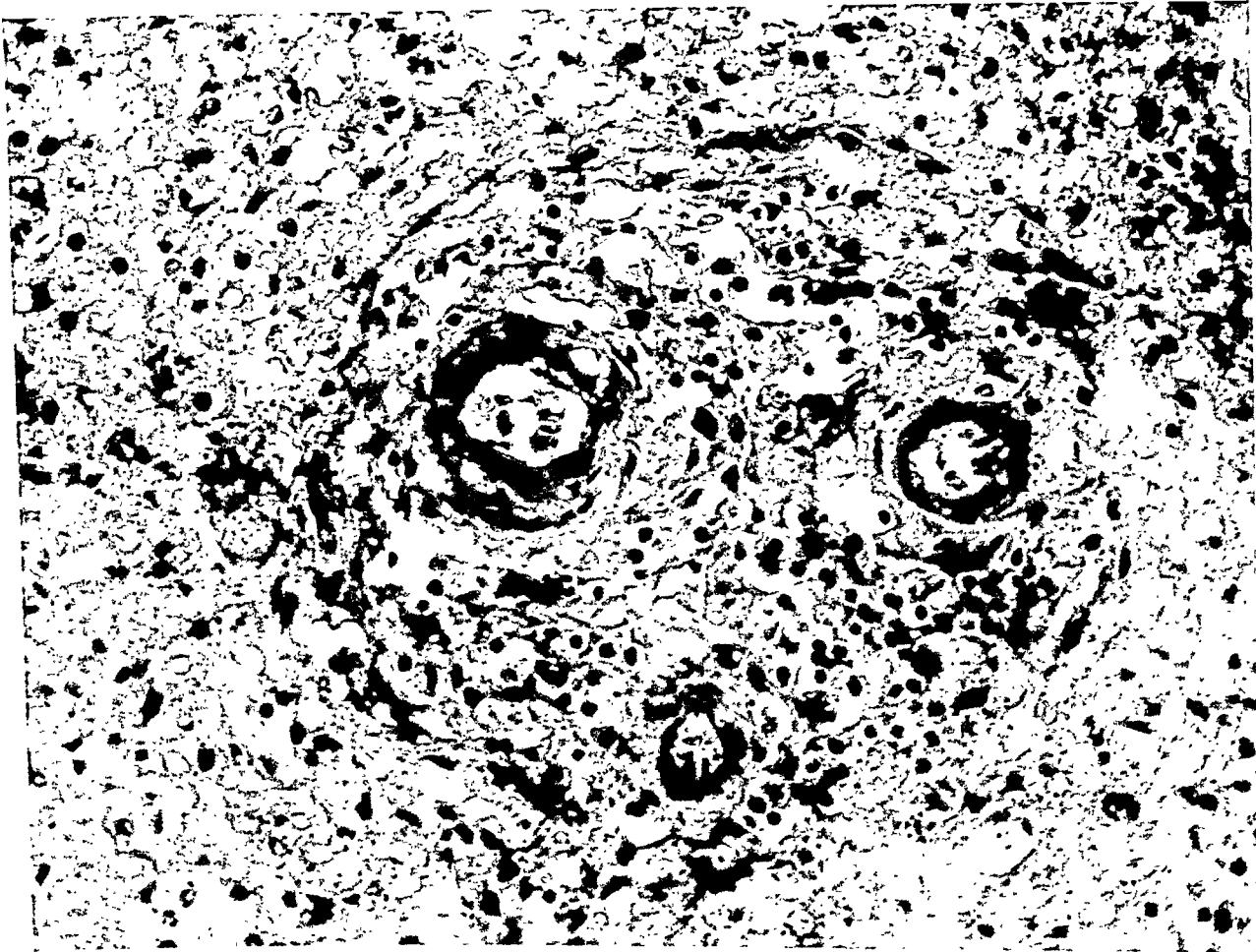
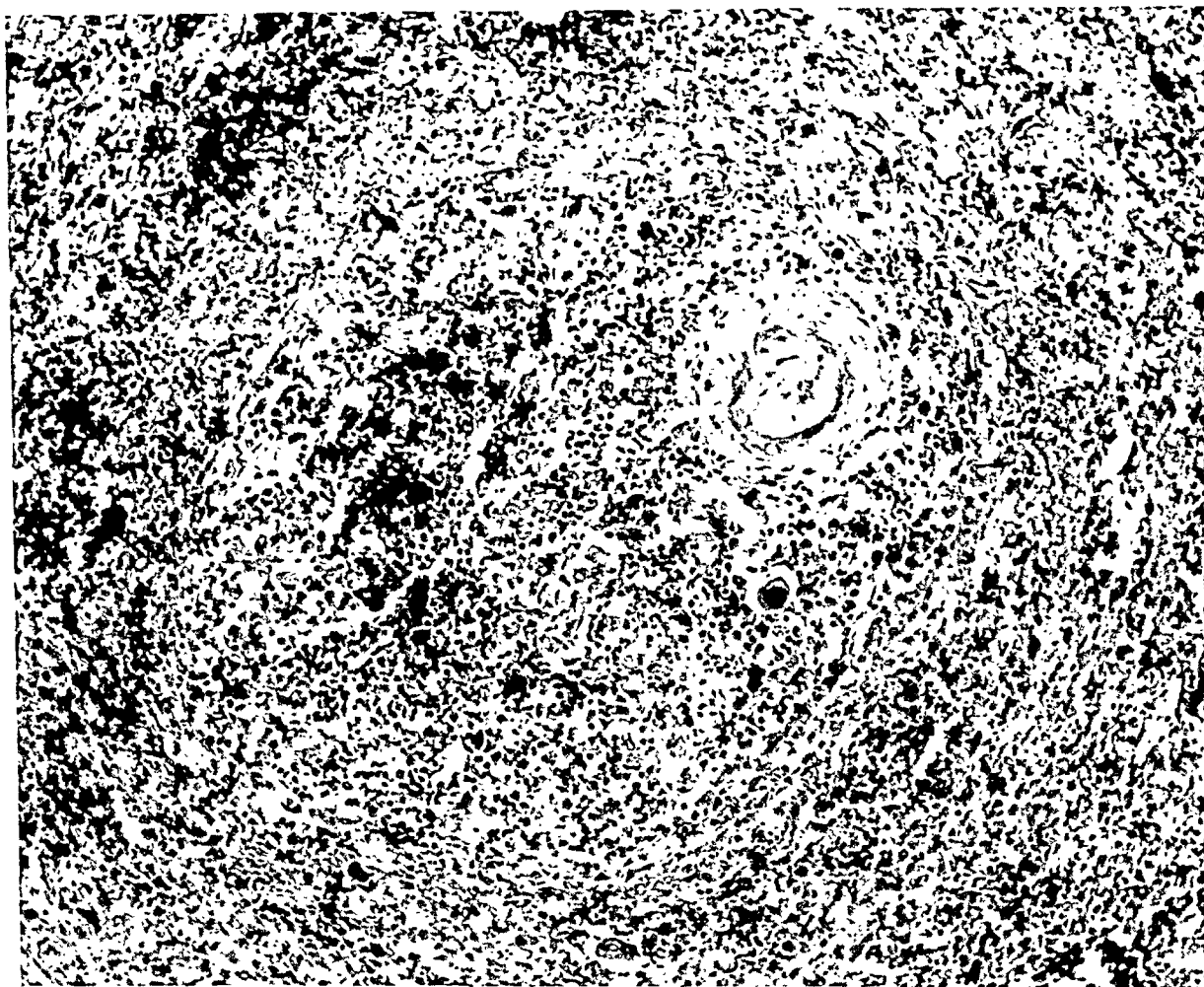


PLATE 131

FIG. 30. Group I. Spleen. Malpighian corpuscle. Paucity of lymphocytes. Giant cell resembling Reed-Sternberg cell. Hemorrhage in and about malpighian follicle. K-3. Sakuma. Male, 15 years of age. Approximately 1000 yds. Died on the fifth day. A.I.P. neg. HM 107.  $\times 165$ .

FIG. 31. Group I. Spleen. Erythrophagocytosis. Atypical large mononuclear cells. From the same patient as Figure 30. A.I.P. neg. HM 120.  $\times 750$ .

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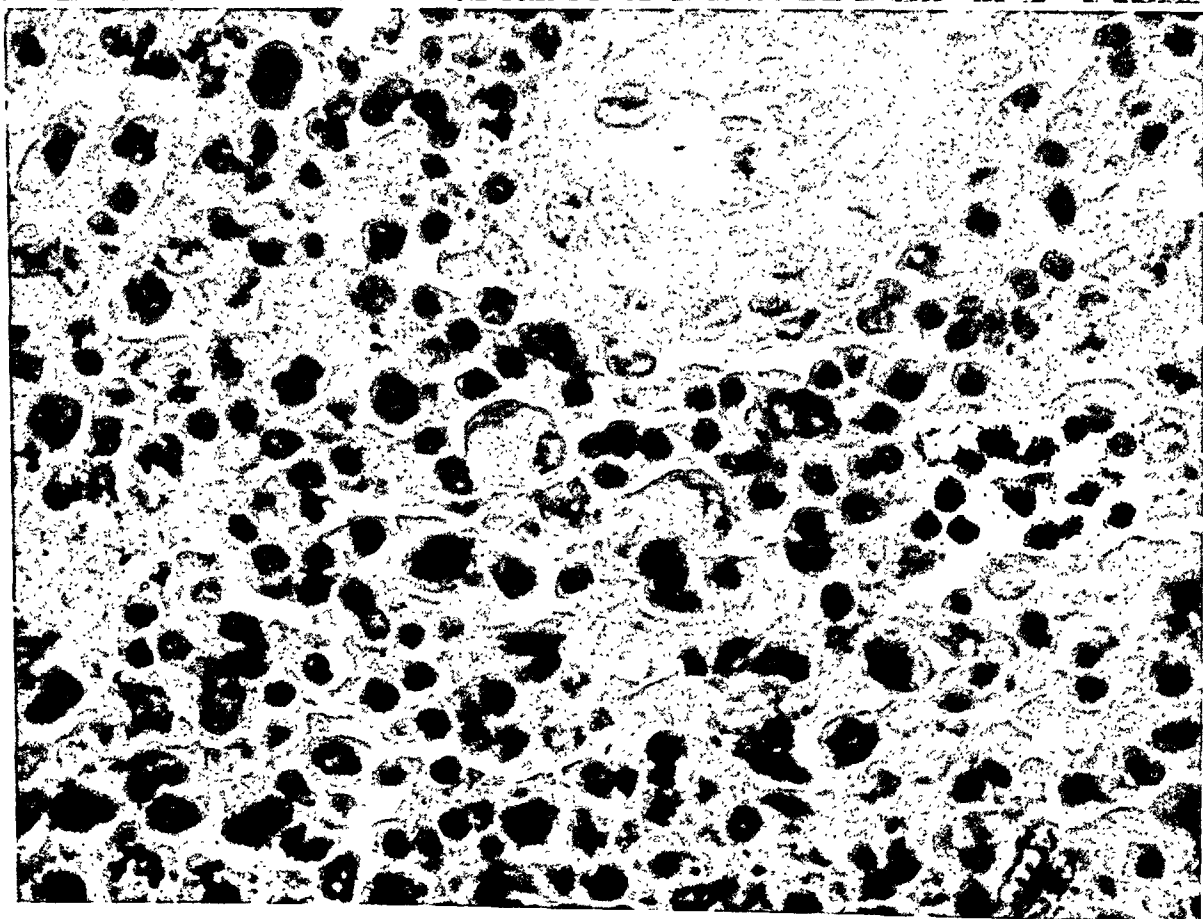
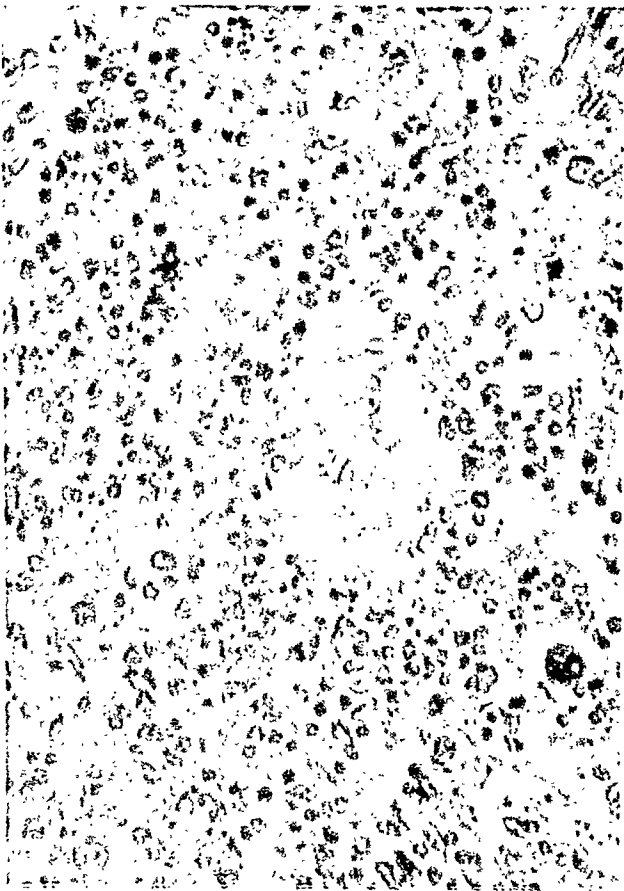


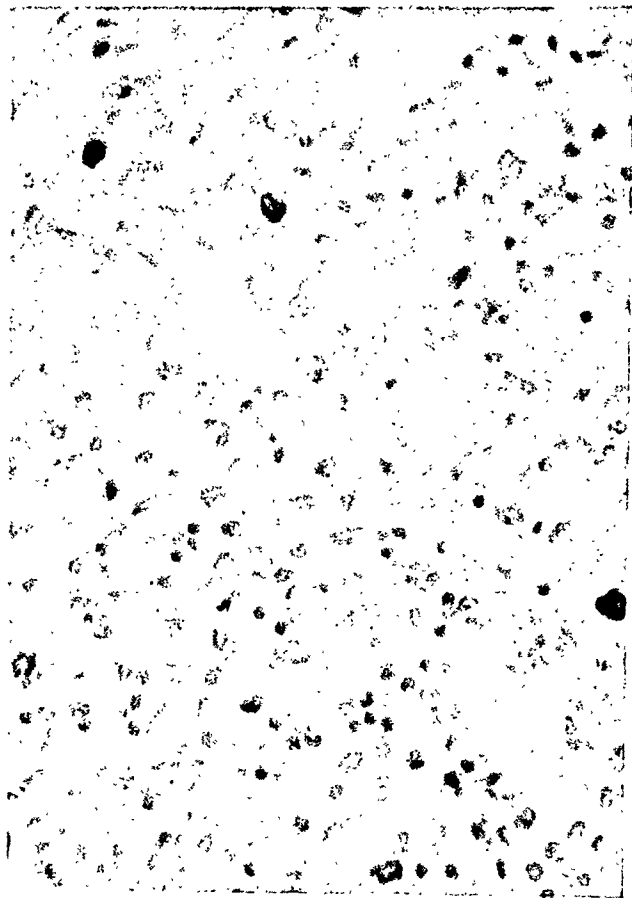
PLATE 132

- FIG. 32. Group I. Spleen. Knot of reticulum cells at center. Large atypical mononuclear elements. Group of minute nuclei. From the same patient as Figure 30. A.I.P. neg. HM 121 (K).  $\times 350$ .
- FIG. 33. Group II. Lymph node. Almost total disappearance of lymphocytes. Skeleton of reticular cells. Numerous mast cells. Giemsa's stain, K-28. Kawaura. Male, 23 years of age. Approximately 1000 yds. Died on the 26th day. A.I.P. neg. HM 295.  $\times 500$ .
- FIG. 34. Group III. Femur. Red gelatinous and gray-red cellular marrow in upper portion of shaft. Gelatinous and fatty yellow marrow in distal portion of the bone. From the same patient as Figure 70. A.I.P. neg. HS 334 (K).
- FIG. 35. Group I. Marrow from long bone. Reticulum cells, plasmacytoid elements, and some large cells intermediate in appearance between the two. No normal hemopoietic tissue. Erythrophagocytosis by some of the reticular elements. This is the earliest histologic specimen of bone marrow available. By the criteria employed in the text it would be classified "type A," marked hypoplasia. K-5. Yano. Male, 39 years of age. Approximately 1000 yds. Died on the sixth day. A.I.P. neg. HM 131.  $\times 650$ .

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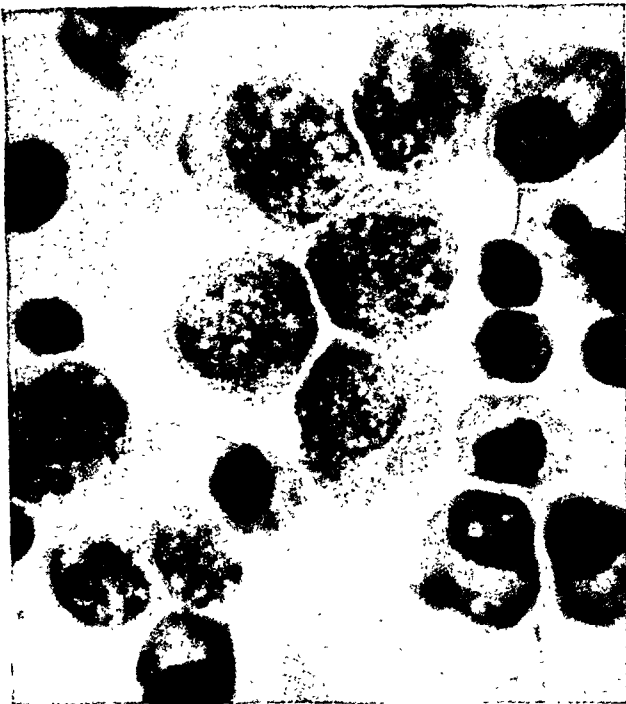
Pathology of Atomic Bomb Casualties



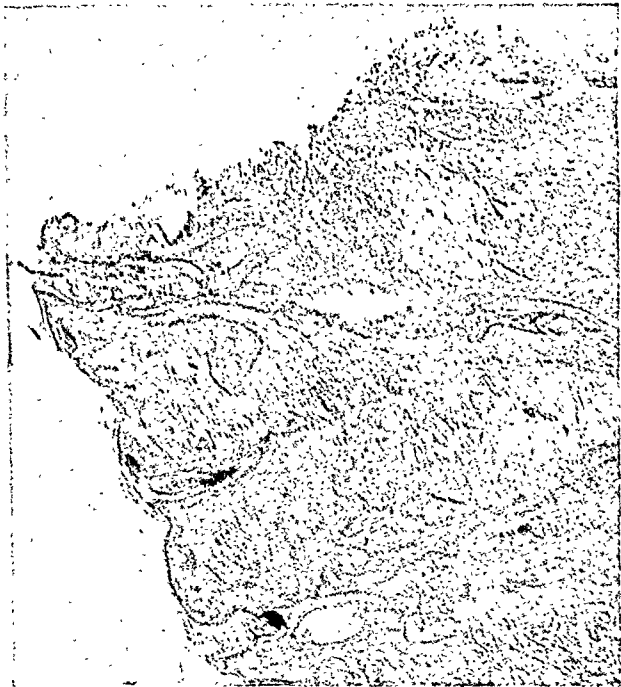
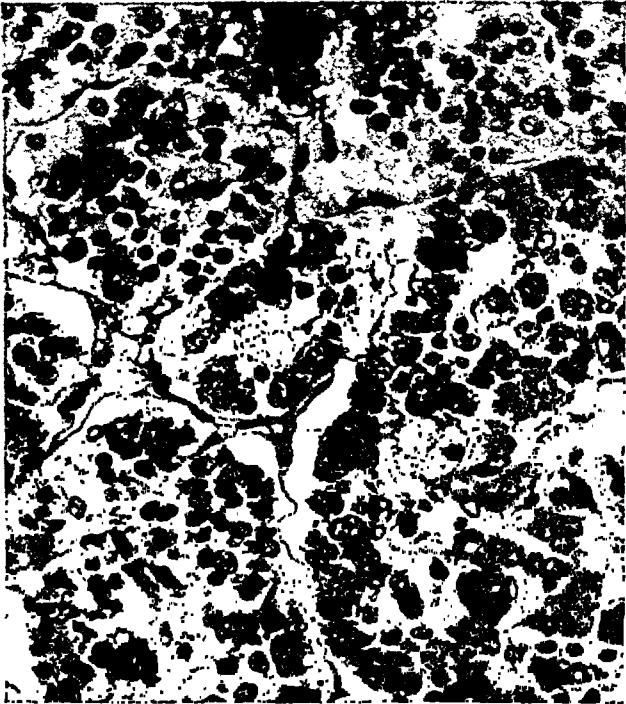
PLATE 133

- FIG. 36. Group II. Smear of bone marrow. Reticulum cells, myelocytes with azurophilic granules, and cells transitional between the two. These transitional forms suggest a direct origin of myelocytes from reticulum cells in these rapidly regenerating marrows. Numerous plasma cells and forms transitional between them and the reticulum cells. Blast cells are rare in this smear. Wright-Giemsa stain. K-43. Horinouchi. Male, 33 years of age. Approximately 1000 yds. Died on the 32nd day. A.I.P. neg. HM 320 (K).  $\times 800$ .
- FIG. 37. Group II. Pituitary body. Vacuolated large basophilic cells ("castration cells"). Hyperplasia of basophilic cells. K-42. Moriseko. Male, 27 years of age. Approximately 1000 yds. Died on the 31st day. A.I.P. neg. HM 298.  $\times 400$ .
- FIG. 38. Group III. Scalp. Atrophy of hair follicles and associated sebaceous glands. There is no notable change in the sweat glands. For details see Figure 141. K-14. Yamamoto. Male, 25 years of age. Approximately 1000 yds. Died on the 47th day. A.I.P. neg. HM 280 (K).  $\times 22$ .
- FIG. 39. Group III. Testis. Disappearance of germinal epithelium and its derivatives. Thickening of basement membranes of the tubules by deposition of finely fibrillar collagenous material within the old basement membrane. Deposits of refractile acidophilic material beneath the endothelium of the blood vessels in the interstitium. K-14. Yamamoto. Male, 25 years of age. Approximately 1000 yds. Died on the 47th day. A.I.P. neg. HM 211.  $\times 115$ .

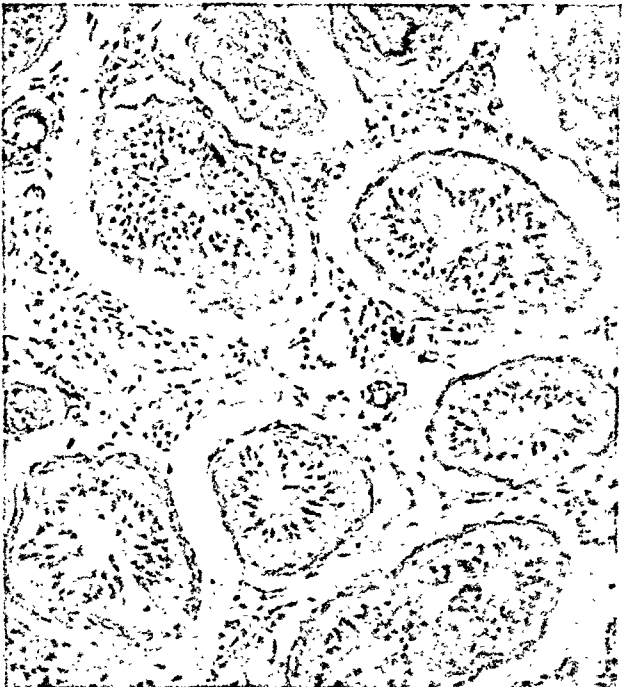
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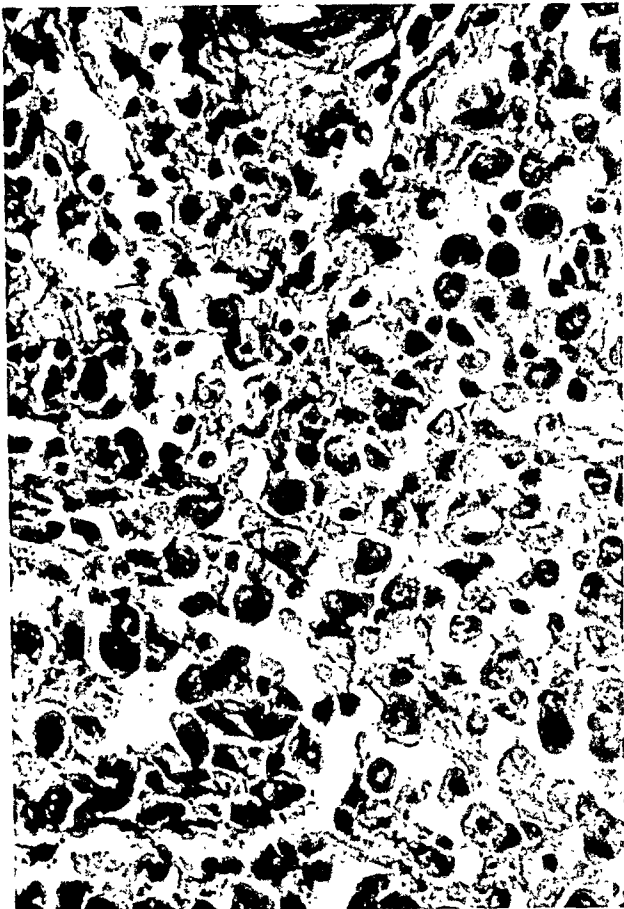
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Pathology of Atomic Bomb Casualties

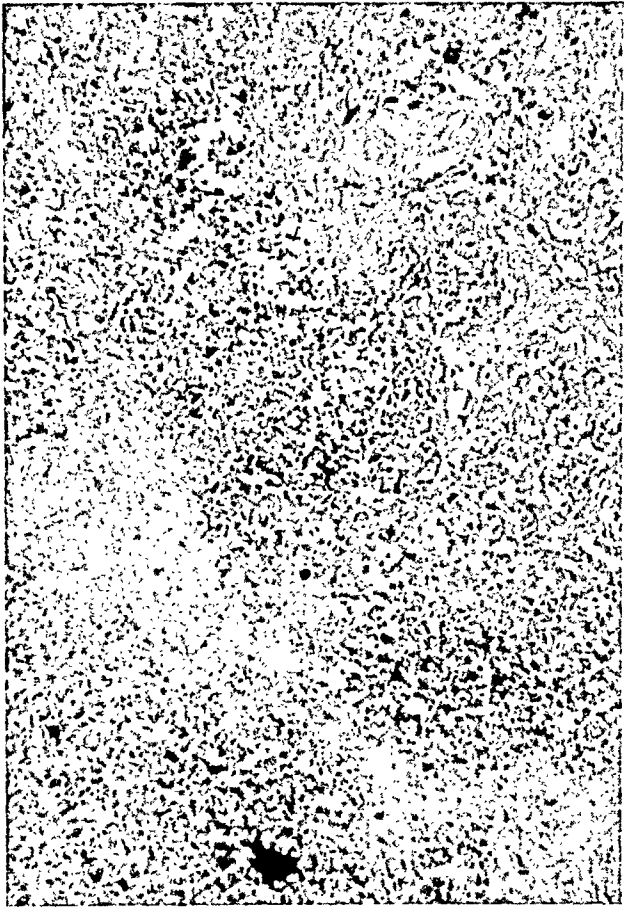
PLATE 134

- FIG. 40. Group I. Spleen. Tissue in neighborhood of central arteriole. Only rare lymphocytes are present. Numerous large atypical mononuclear elements, some in mitosis. K-9. Sakamoto. Male, 25 years of age. Approximately 1500 yds. Died on the eighth day. A.I.P. neg. HM 138.  $\times 400$ .
- FIG. 41. Group II. Spleen. Condensation of reticulum cells about central arteries of malpighian corpuscles. K-35. Takahashi. Male, 31 years of age. Approximately 1000 yds. Died on the 28th day. A.I.P. neg. HM 223.  $\times 120$ .
- FIG. 42. Group II. Spleen. Malpighian corpuscle. Almost total disappearance of typical lymphocytes. A few small and large plasmacytoid elements remain. Deposition of hyaline, homogeneous, acidophilic, refractile material beneath endothelium of central arteriole. Slight, if any, evidence of proliferative activity of the reticulum cells. K-47. Naka. Female, 35 years of age. Approximately 800 yds. Died on the 18th day. A.I.P. neg. HM 247.  $\times 135$ .
- FIG. 43. Group II. Spleen. Atrophy of lymphoid tissue. Atypical large mononuclear elements. Hyaline changes in walls of central arteries. K-121. Sakanishi. Male, 45 years of age. Approximately 1000 yds. Died on the 24th day. A.I.P. neg. HM 306.  $\times 400$ .

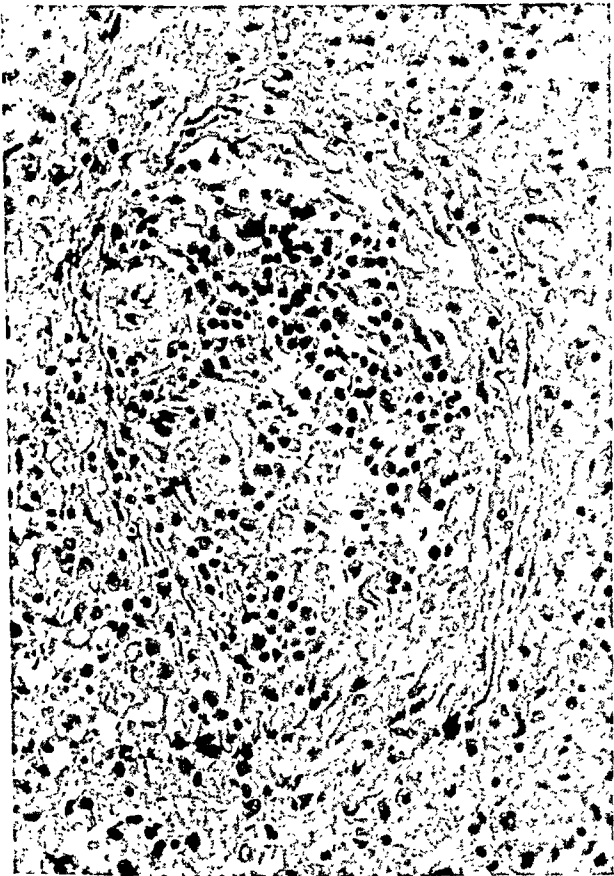
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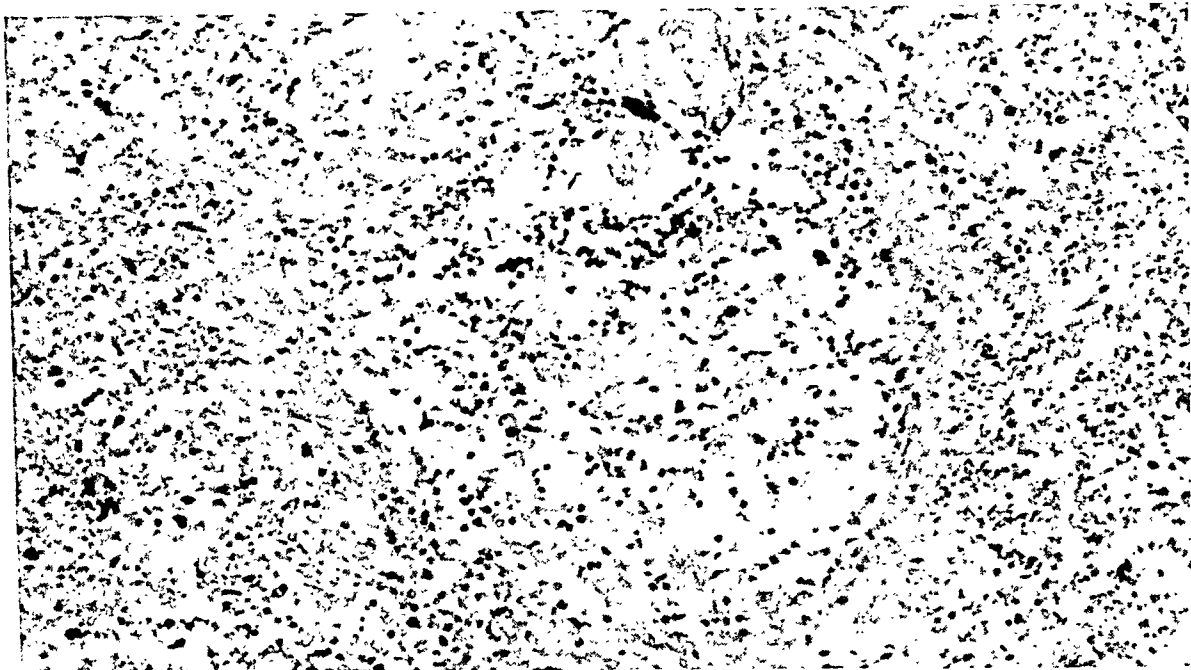
PLATE 135

- FIG. 44. Group I. Lymph node. Collapsed reticular skeleton supporting a very few mature lymphocytes. K-98. Tamai. Male, 19 years of age. Distance unknown. Died on the tenth day. A.I.P. neg. HM 282.  $\times 160$ .
- FIG. 45. Group II. Spleen. Condensation of syncytial masses of reticulum cells about a regenerating malpighian corpuscle. Giemsa's stain. From the same patient as Figure 41. A.I.P. neg. HM 224.  $\times 235$ .
- FIG. 46. Group II. Spleen. Condensation of syncytial reticulum cells at margin of regenerating malpighian corpuscle. Giemsa's stain. Enlargement of a portion of Figure 45. A.I.P. neg. HM 225.  $\times 400$ .

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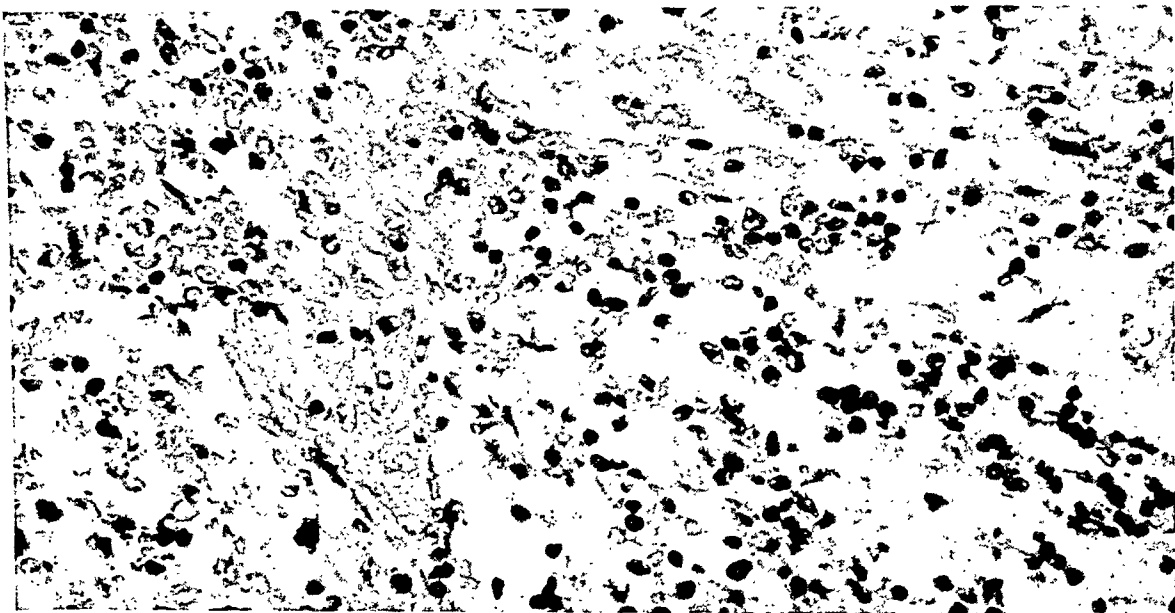
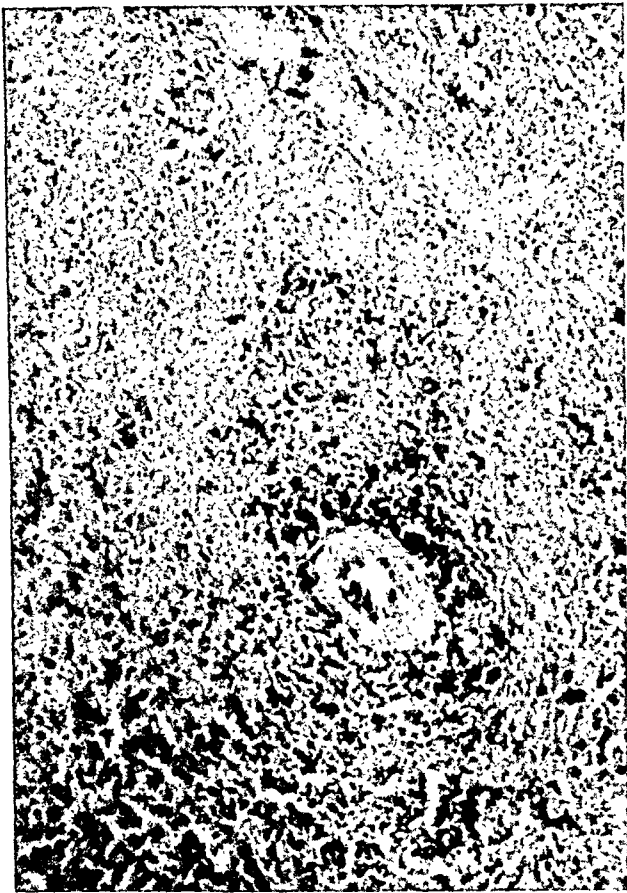


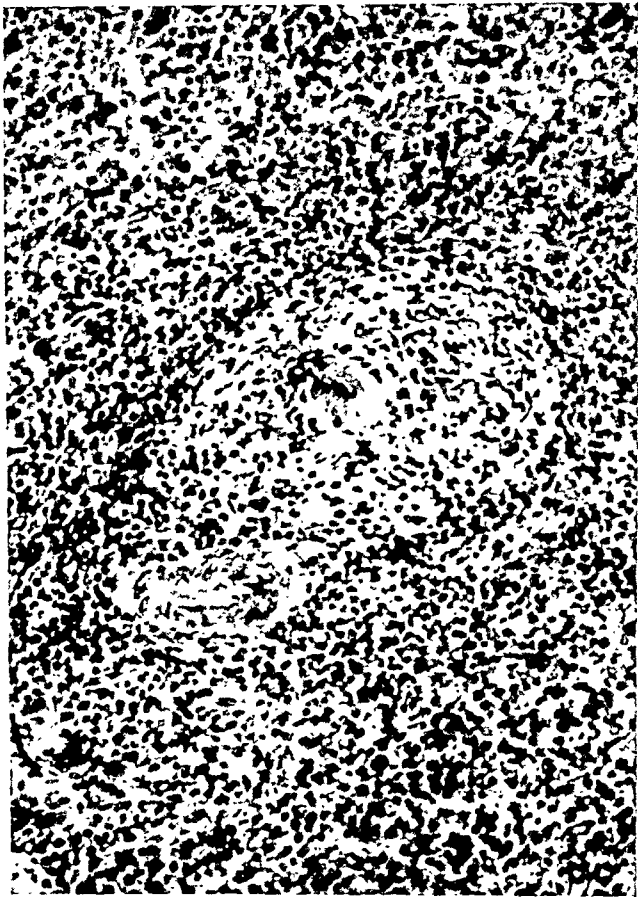
PLATE 136

- FIG. 47. Group II. Spleen. Condensation of syncytial reticulum at periphery of regenerating malpighian corpuscle. Beginnings of germinal center. K-38. Kamihara. Male, 22 years of age. Approximately 1000 yds. Died on the 29th day. A.I.P. neg. HM 226.  $\times 145$ .
- FIG. 48. Group III. Spleen. Germinal center in malpighian corpuscle. K-50. Kijima. Male, 31 years of age. Approximately 1000 yds. Died on the 100th day. A.I.P. neg. HM 267.  $\times 200$ .
- FIG. 49. Group II. Lymph node. Absence of germinal centers. Mature small lymphocytes are few. Numerous large lymphocytes, cells with the structure of lymphoblasts, and some elements intermediate in appearance between these and the elements of the reticulum. K-104. Yoshitomi. Male, 14 yrs. of age. Approximately 1000 yds. Died on the 21st day. A.I.P. neg. HM 286.  $\times 115$ .
- FIG. 50. Group II. Lymph node. Enlargement of a portion of Figure 49. A.I.P. neg. HM 285.  $\times 400$ .

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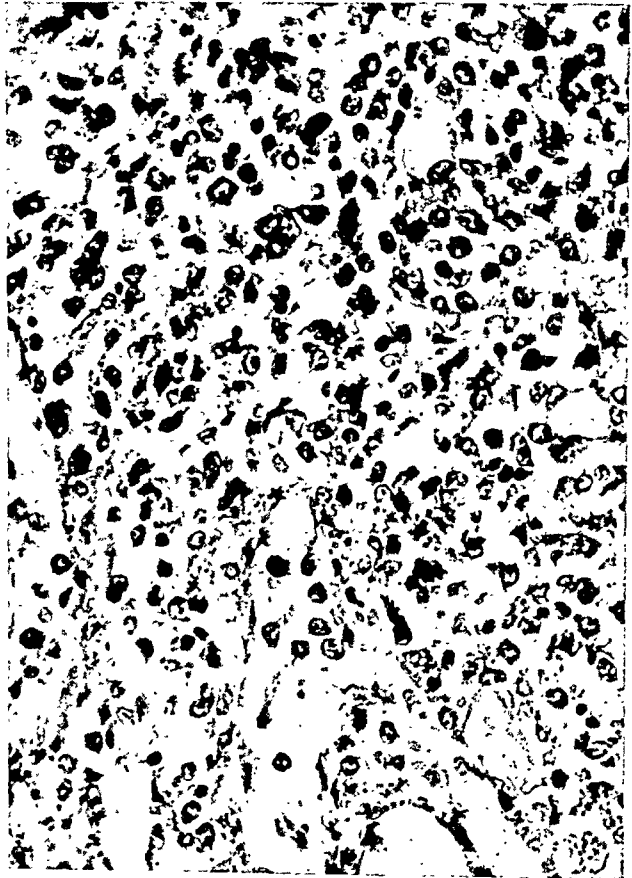
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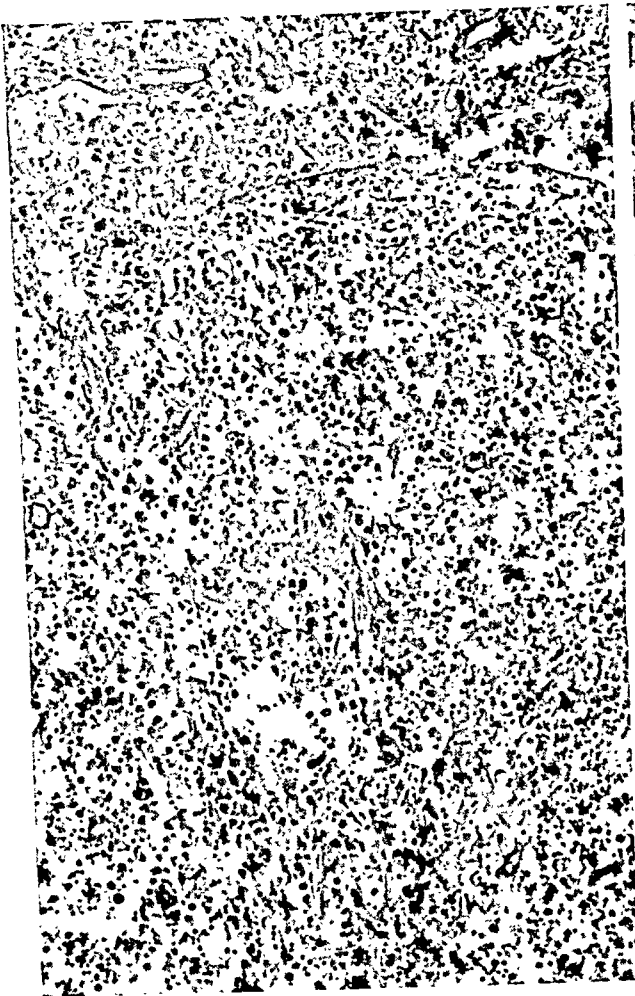
Pathology of Atomic Bomb Casualties



PLATE 137

- FIG. 51. Group II. Lymph node. General view. Absence of secondary follicles. Very few small lymphocytes remain. Numerous plasma cells and larger cells intermediate in appearance between them and the elements of the reticulum. Large mononuclear cells with vacuolated cytoplasm in peripheral sinusoid. Thickening of capsule by granulation tissue. K-30. Nagashima. Male, 23 or 28 years of age (variously stated). Approximately 1000 yds. Died on the 26th day. A.I.P. neg. HM 213.  $\times 120$ .
- FIG. 52. Group II. Lymph node. Enlargement of a portion of Figure 51, including a subcapsular sinusoid and the underlying parenchyma. A.I.P. neg. HM 214.  $\times 430$ .
- FIG. 53. Group II. Lymph node. Tissue near edge of focus of necrosis. Large mononuclear elements with folded nuclei, probably atypical reticulum cells. Occasional plasma cells. Hemorrhage. K-28. Kawaura. Male, 23 years of age. Approximately 1000 yds. Died on the 26th day. A.I.P. neg. HM 152.  $\times 450$ .
- FIG. 54. Group III. Lymph node. Enormous atypical cells resembling Reed-Sternberg cells. K-82. Oku. Female, 15 years of age. Approximately 1200 yds. Died on the 42nd day. A.I.P. neg. HM 301.  $\times 400$ .

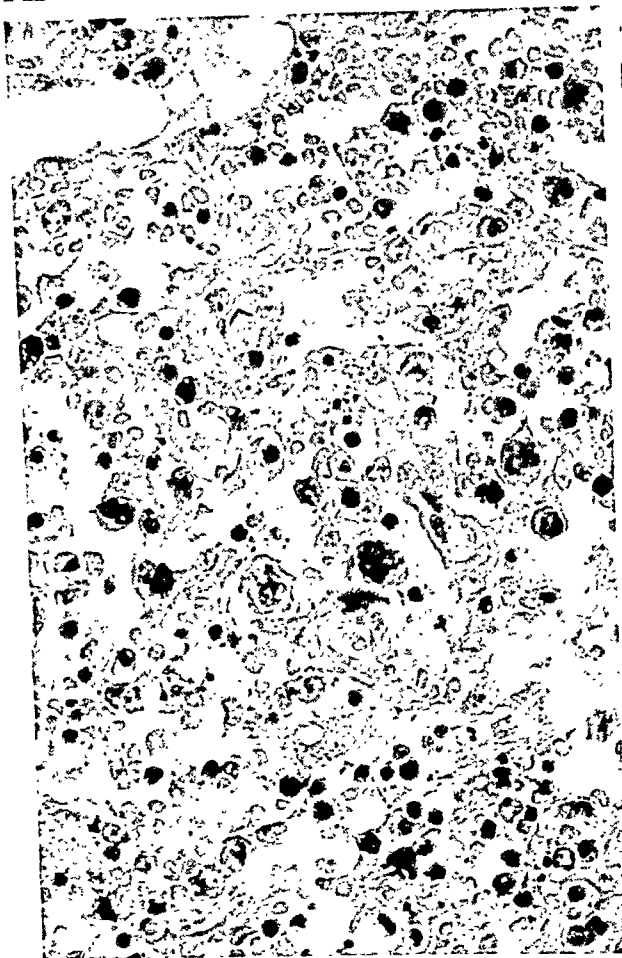
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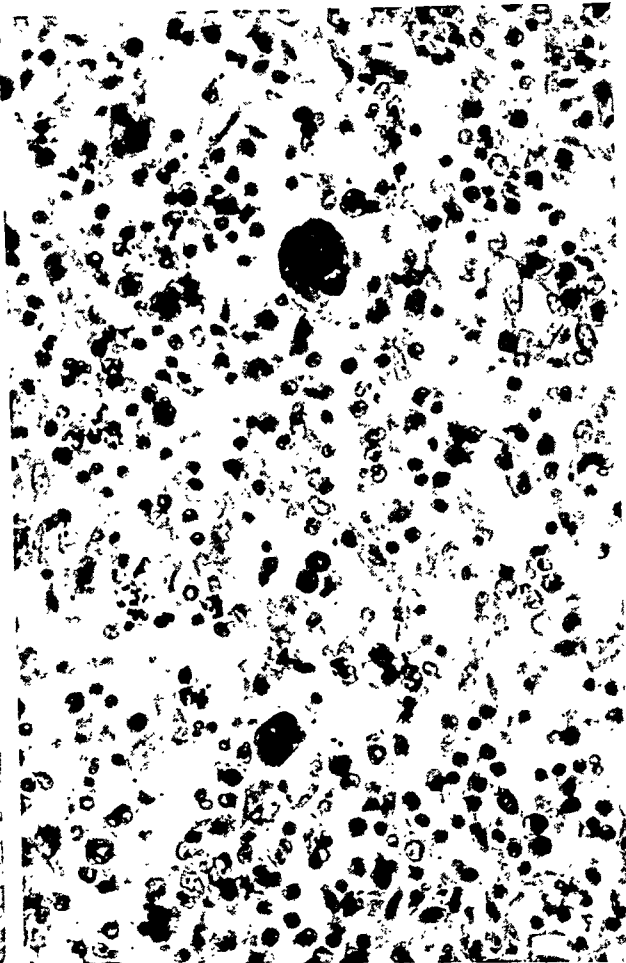
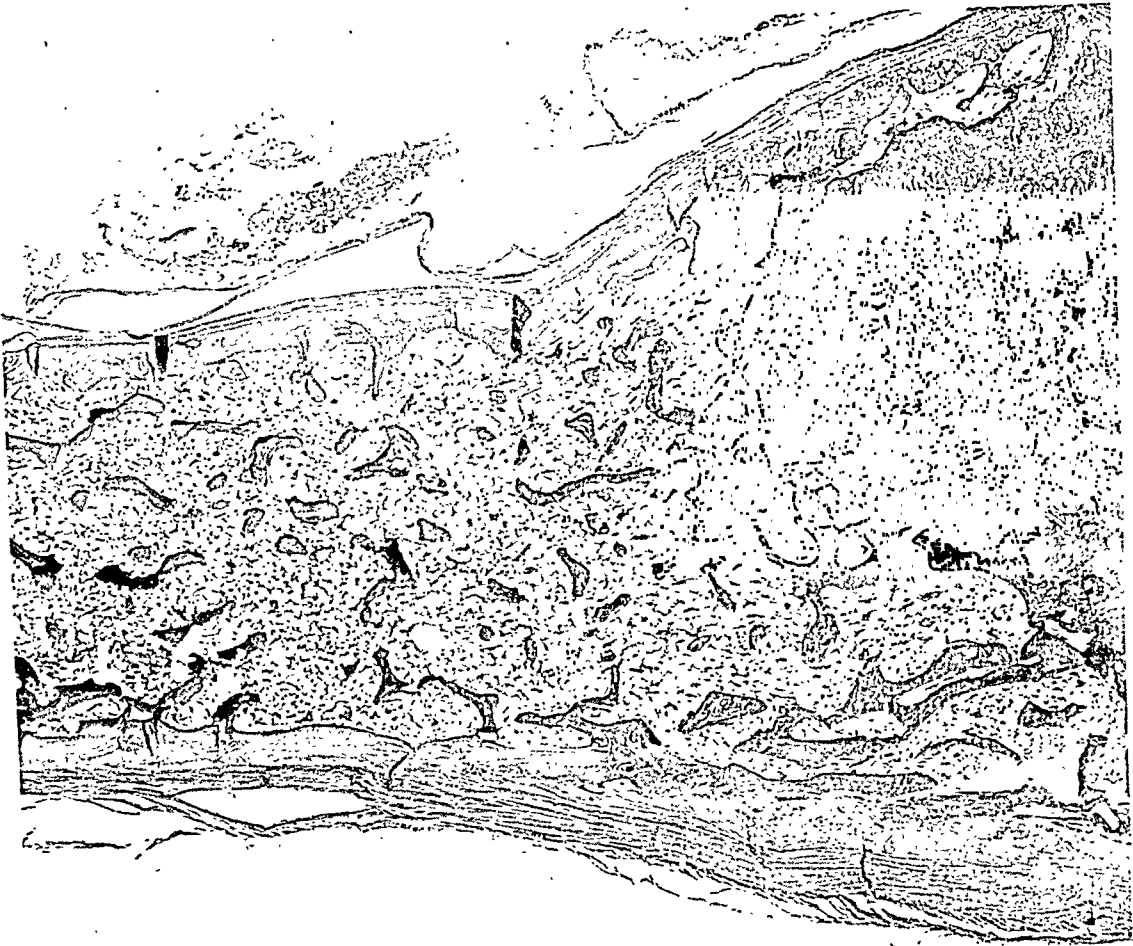


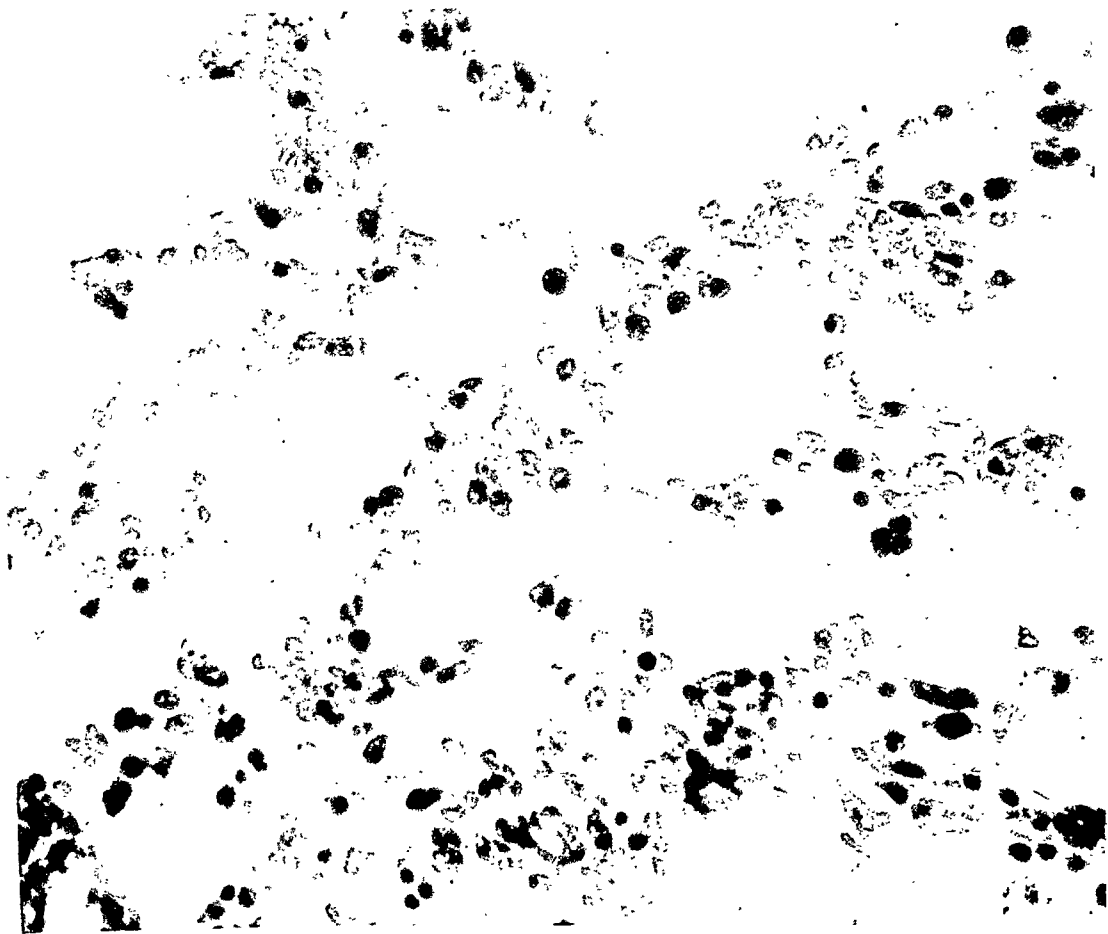
PLATE 138

- FIG. 55. Group II. Bone marrow, rib. Extreme hypoplasia, "type A" marrow. K-119. Nagado. Female, 26 years of age. Approximately 1300 yds. Died on the 23rd day. A.I.P. neg. HM 340.  $\times 13$ .
- FIG. 56. Group II. Bone marrow, rib. Hypoplasia, "type A" marrow. Reticulum cells and plasmacytoid elements. Giemsa's stain. K-39. Takeuchi. Male, 29 years of age. Approximately 1000 yds. Died on the 29th day. A.I.P. neg. HM 184.  $\times 180$ .

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# PLATE 139

FIG. 57. Group III. Rib. Costochondral junction. Hyperplasia of bone marrow, "type C" marrow. K-133. Nagai. Male, 9 years of age. Approximately 500 yds. Died on the 54th day. A.I.P. neg. HM 337.  $\times 13$ .

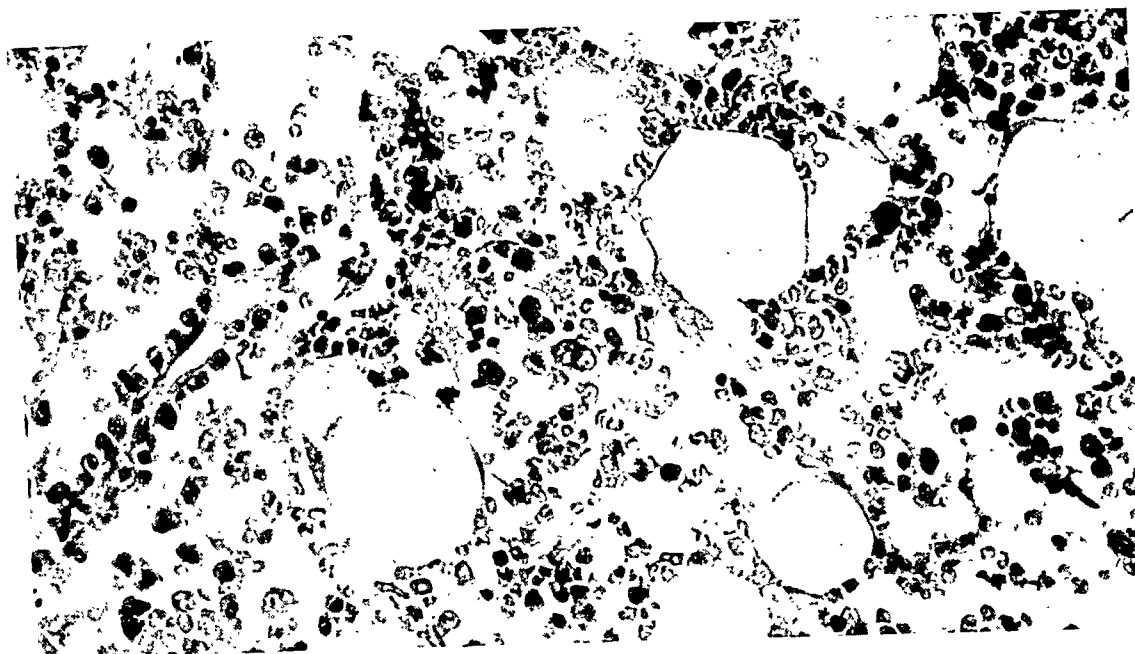
FIG. 58. Group I. Bone marrow, sternum. Hypoplasia. Plasma cells arranged adventitiously in relation to the sinusoids. Some large cells intermediate in appearance between the plasma cells and elements of the reticulum. This is the earliest histologic specimen of marrow available from a bone in which there is usually active hemopoiesis. Its classification is "type A," marked hypoplasia. K-98. Tamai. Male, 19 years of age. Distance unknown. Died on the tenth day. A.I.P. neg. HM 283.  $\times 400$ .

FIG. 59. Group I. Bone marrow. Many plasma cells. Focal hyperplasia of reticulum. Some cells intermediate in appearance between plasma cells and elements of the reticulum. The atypical plasmacytoid differentiation is remarkable. No erythropoietic tissue or megakaryocytes are in evidence. This marrow is classified as "type B," marked focal reticulum hyperplasia. K-100. Ueki. Female, 32 years of age. Distance unknown. Died on the twelfth day. A.I.P. neg. HM 284.  $\times 400$ .

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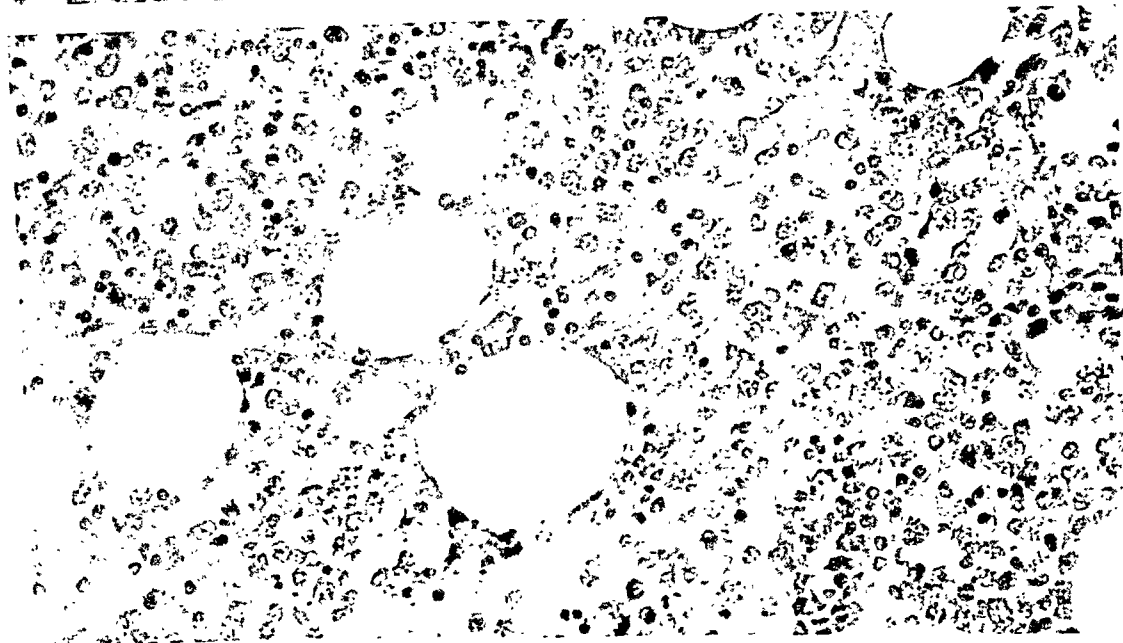
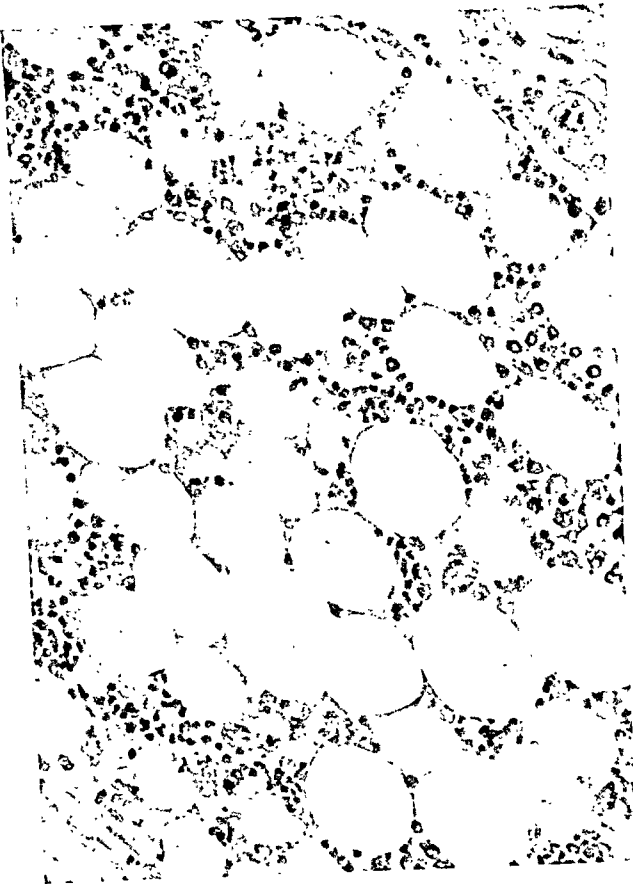


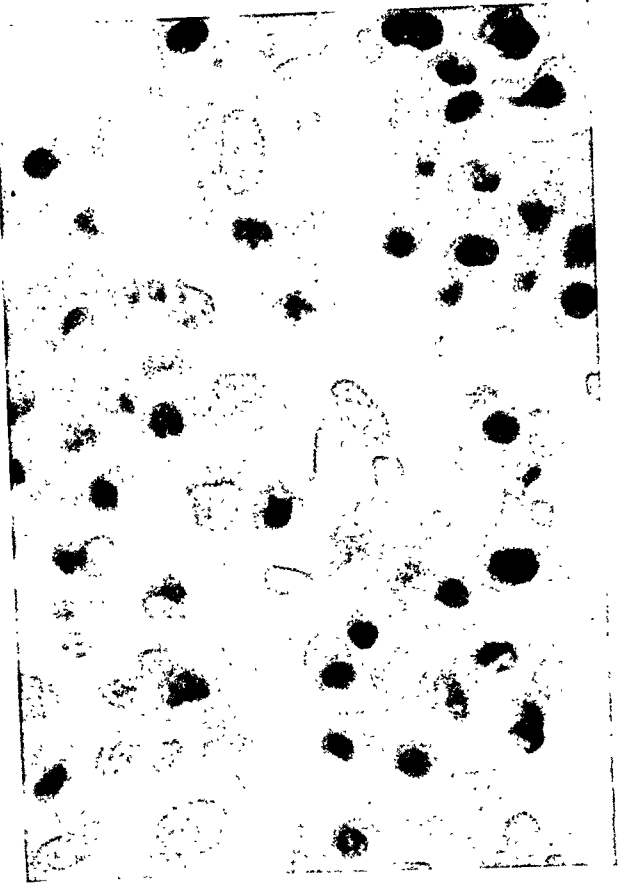
PLATE 140

- FIG. 60. Group II. Bone marrow (femoral). General view. Groups of reticulum cells, lymphocytes, and plasmacytoid elements. Islets of erythropoietic tissue persist. "Type A" marrow. K-25. Shintaku. Male, age unknown. Approximately 1000 yds. Died on the 25th day. A.I.P. neg. HM 195.  $\times 230$ .
- FIG. 61. Group II. Bone marrow. Femoral. "Type A" marrow. Reticulum and plasma cells. Erythrophagocytosis. K-28. Kawaura. Male, 23 years of age. Approximately 1000 yds. Died on the 26th day. A.I.P. neg. HM 200.  $\times 1000$ .
- FIG. 62. Group II. Vertebral marrow. Hyperplasia of reticulum cells. "Type B" marrow. K-22. Michihara. Male, 17 or 23 years of age (variously stated). Approximately 1000 yds. Died on the 25th day. A.I.P. neg. HM 147.  $\times 450$ .
- FIG. 63. Group II. Bone marrow. Reticulum cell hyperplasia. "Type B" marrow. Prominent nucleoli in some cells. Some cells are still spindle-shaped and others have become rounded. Some plasma cells are present. Giemsa's stain. K-41. Takano. Male, 23 years of age. Approximately 1000 yds. Died on the 30th day. A.I.P. neg. HM 324.  $\times 1020$ .
- FIG. 64. Group II. Reticulum hyperplasia. Giant cell, possibly immature megakaryocyte. From the same patient as Figure 63. A.I.P. neg. HM 323.  $\times 1020$ .

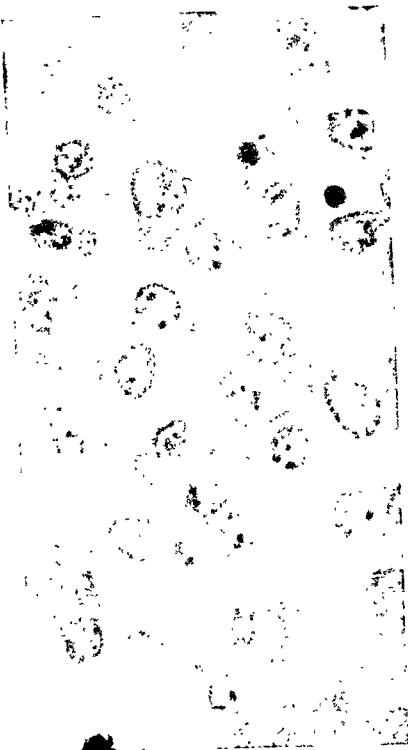
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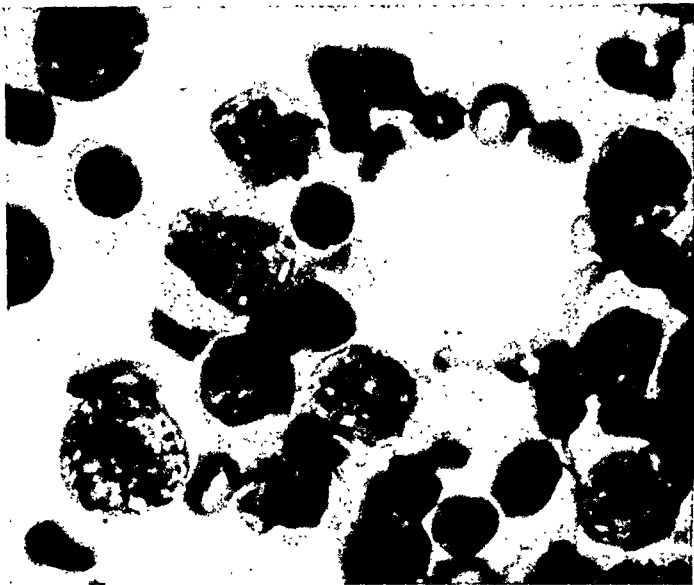
Pathology of Atomic Bomb Casualties



PLATE 141

- FIG. 65. Group II. Bone marrow, sternum. Reticulum cells, young myelocytes, and plasma cells. K-27. Omura. Male, 22 years of age. Approximately 1000 yds. Died on the 26th day. A.I.P. neg. HM 250.  $\times 1000$ .
- FIG. 66. Group II. Bone marrow, vertebral. Streptococci in wall of sinusoid, without local tissue response. Numerous large reticulum cells. Giemsa's stain. K-22. Michihara. Male, 23 or 17 years of age (variously stated). Died on the 25th day. A.I.P. neg. HM 183.  $\times 1100$ .
- FIG. 67. Group II. Bone marrow, rib. Focus of necrosis. Masses of bacteria. Giemsa's stain. K-39. Takeuchi. Male, 29 years of age. Approximately 1000 yds. Died on the 29th day. A.I.P. neg. HM 197.  $\times 230$ .
- FIG. 68. Group II. Bone marrow, rib. Bacilli in large numbers, without cellular reaction. Giemsa's stain. Enlargement of a portion of Figure 67. A.I.P. neg. HM 198.  $\times 1000$ .
- FIG. 69. Group III. Hyperplasia of reticulum. Some cells have more prominent nucleoli than are seen in the typical reticulum cell and may be forms in transition to myelocytes, although the granules in the cytoplasm are not seen. Blast cells are rare. K-118. Inaba. Female, 56 years of age. Approximately 700 yds. Died on the 60th day. A.I.P. neg. HM 316.  $\times 750$ .

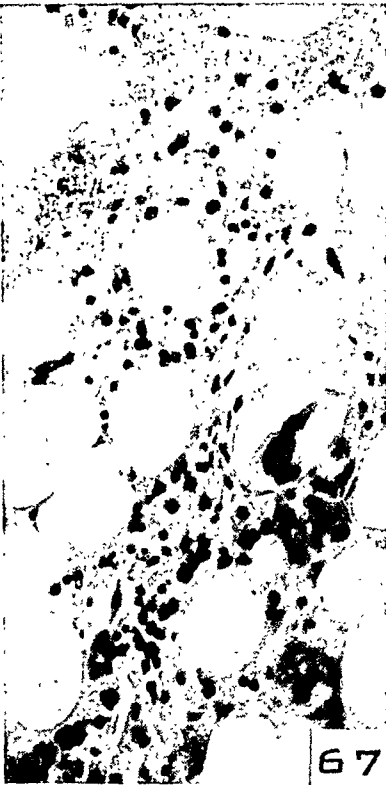
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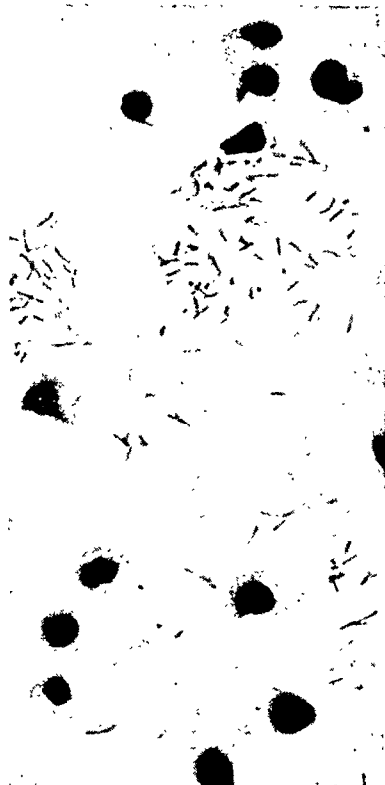
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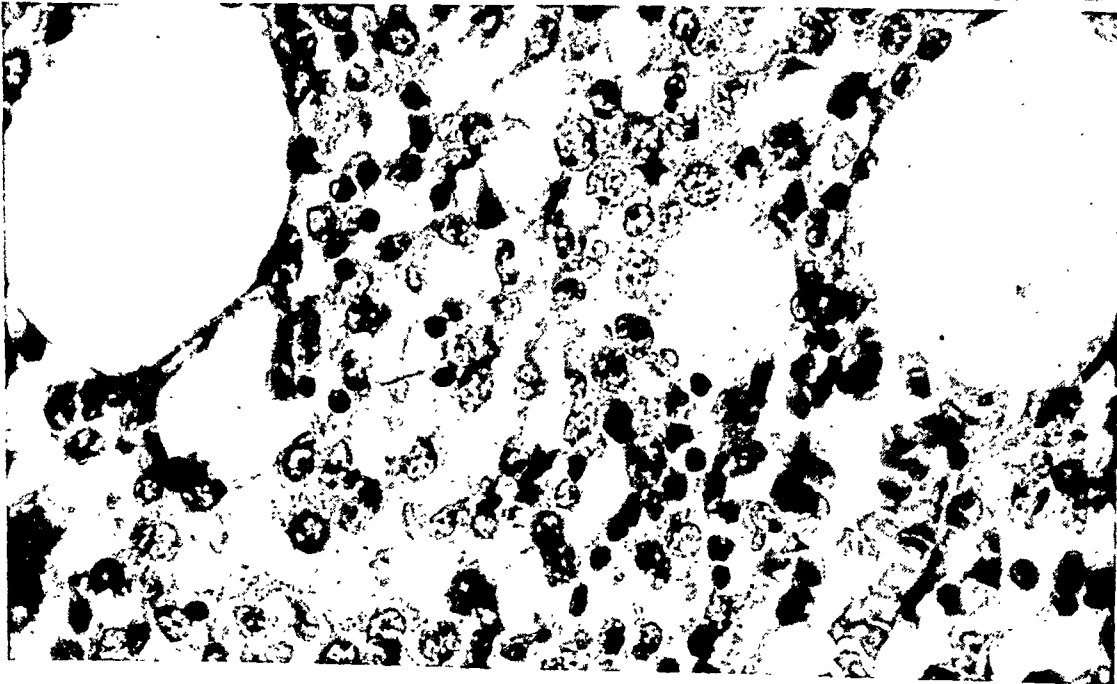


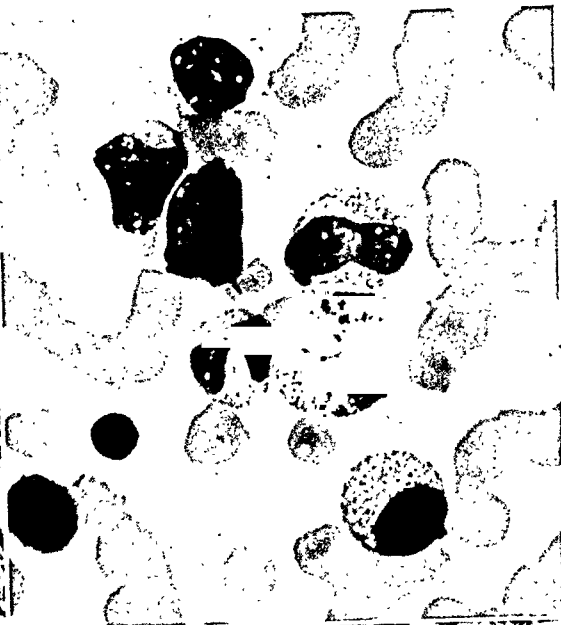
PLATE 142

- FIG. 70. Group III. Bone marrow of rib. Focus of regenerating tissue within the gelatinous marrow. "Type C" marrow. The vertebral marrow in this patient was much more cellular. K-50. Kijima. Male, 31 years of age. Approximately 1000 yds. Died on the 100th day. A.I.P. neg. HM 142a.  $\times 115$ .
- FIG. 71. Group III. Bone marrow smear. Myelocytes and a cell with clear cytoplasm resembling that of a lymphocyte. From the same patient as Figure 70. A.I.P. neg. HM 309.  $\times 800$ .
- FIG. 72. Group III. Bone marrow. Extreme hyperplasia, "Type D" marrow. Myelocytes and metamyelocytes predominate. Small islands of erythropoietic tissue. K-14. Yamamoto. Male, 25 years of age. Approximately 1000 yds. Died on the 47th day. A.I.P. neg. HM 261.  $\times 130$ .
- FIG. 73. Group III. Bone marrow, sternal. Fibrin deposited about megakaryocytes. K-110. Watanabe. Male, 56 years of age. Approximately 1000 yds. Died on the 27th day. A.I.P. neg. HM 287.  $\times 500$ .
- FIG. 74. Group III. Bone marrow, rib. "Gelatinous marrow" showing atrophic fat cells, and granular interstitial material containing large quantities of fibrin. Masson's stain. K-50. Kijima. Male, 31 years of age. Approximately 1000 yds. Died on the 100th day. A.I.P. neg. HM 266.  $\times 400$ .

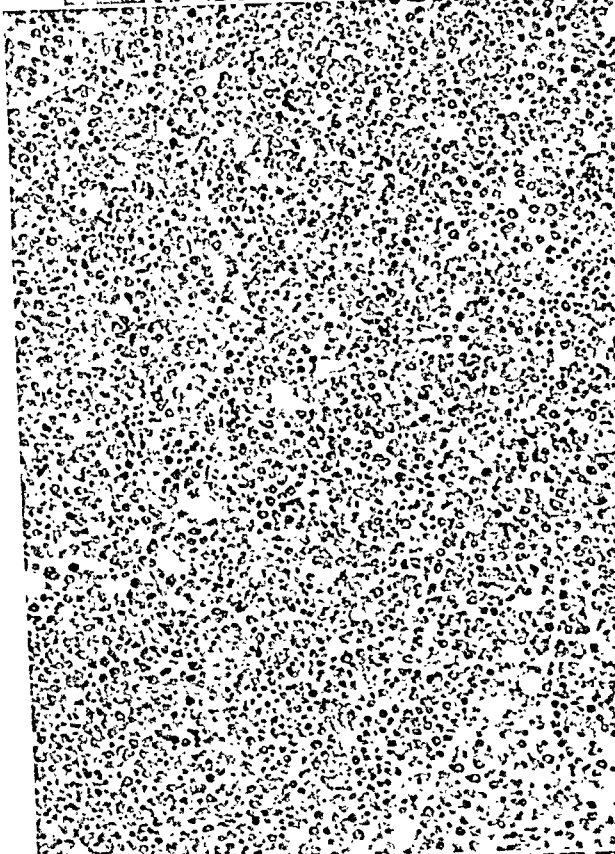
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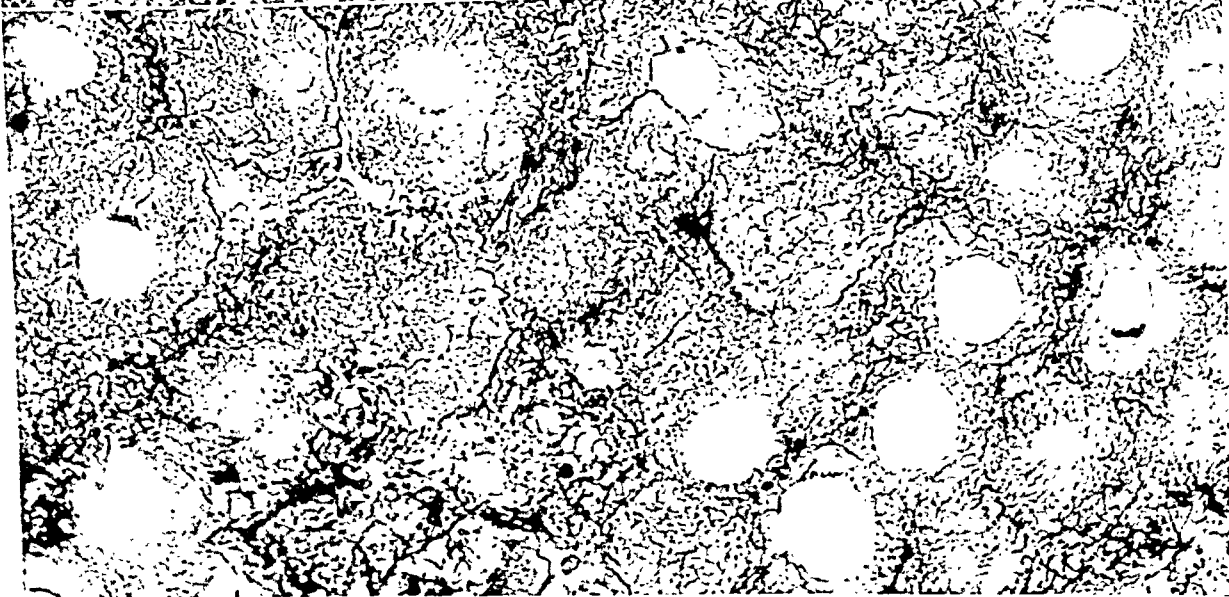
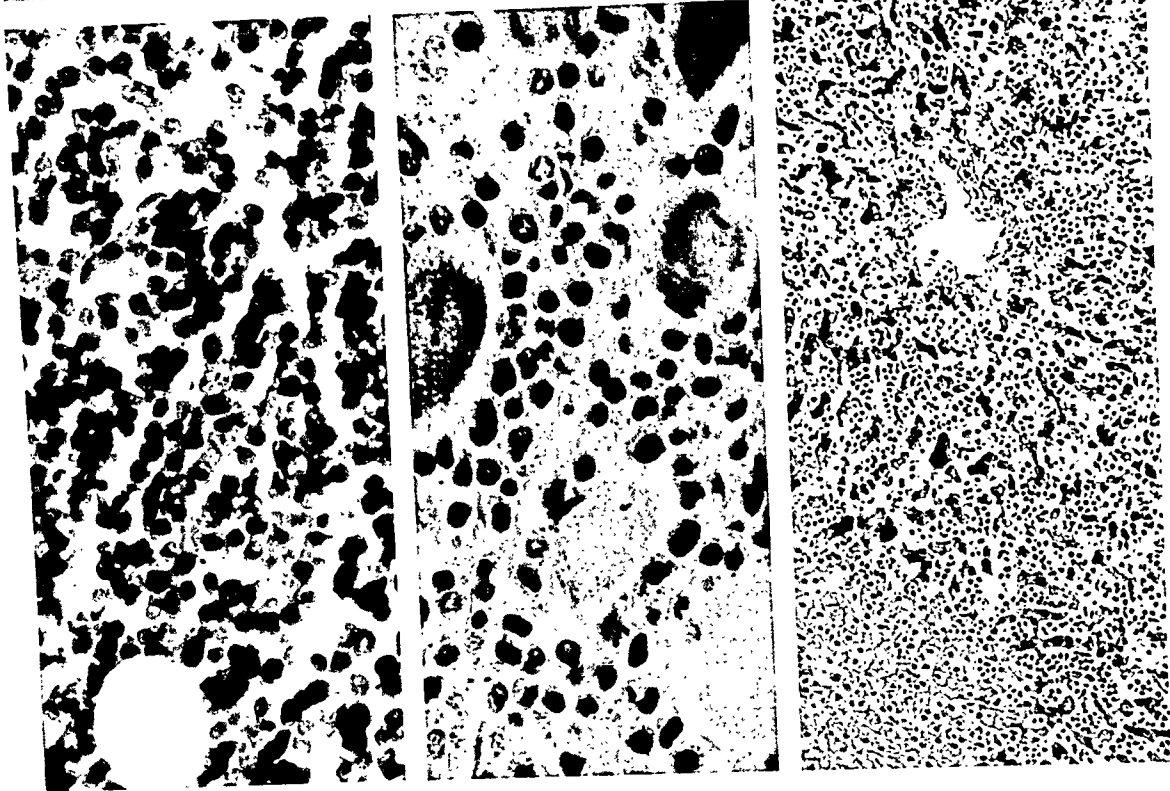
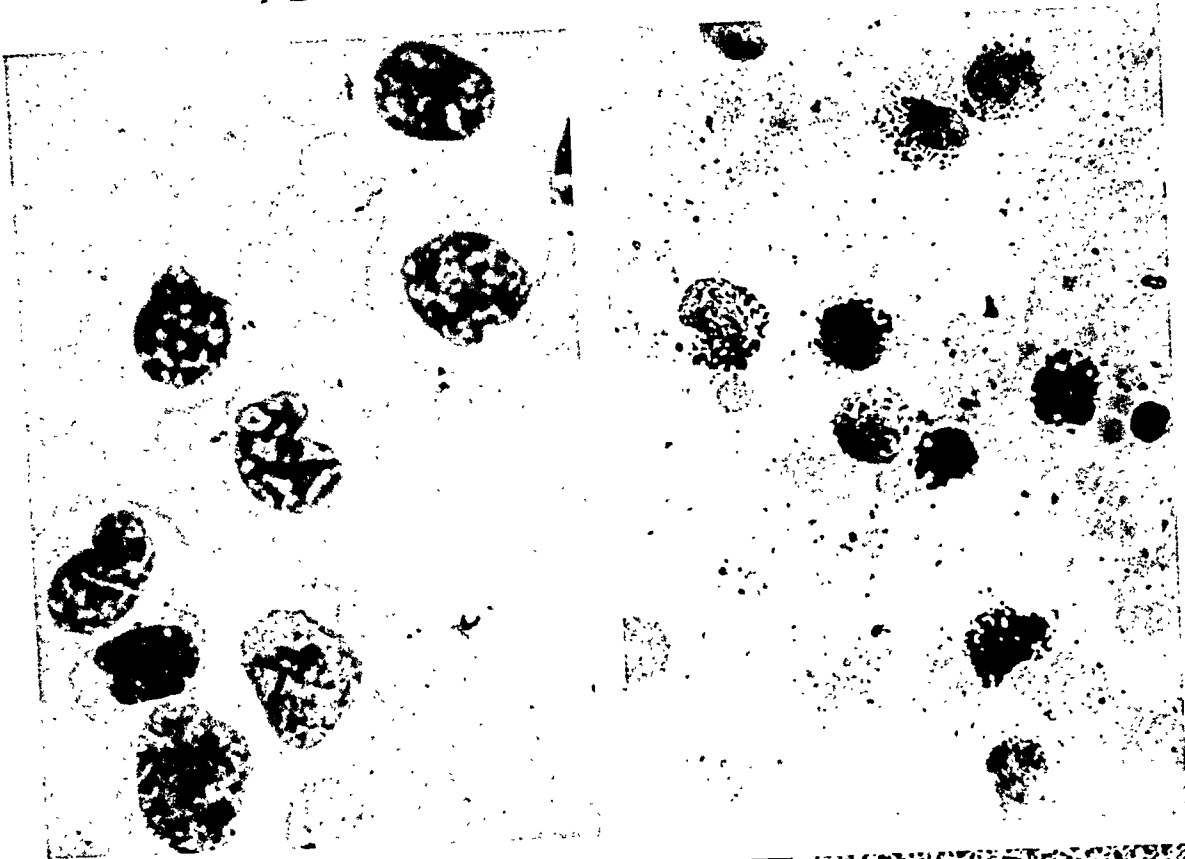


PLATE 143

- FIG. 75. Group III. Peripheral blood film. Cells of monocytic type. K-224. (Nagasaki.) Matsuo. Male, 19 years of age. Approximately 1000 yds. Died on the 97th day. A.I.P. neg. NM 163.  $\times 1100$ .
- FIG. 76. Group III. Supravital blood film of November 16, 1945, showing phagocytosis of carbon by the atypical mononuclear cells. From the same patient as Figure 75. A.I.P. neg NM 158. Copy of Japanese photograph.
- FIG. 77. Group III. Bone marrow (probably from long bone). Large mononuclear cells forming thick septa among the fat cells. From the same patient as Figure 75. A.I.P. neg. NM 164.  $\times 400$ .
- FIG. 78. Group III. Striated muscle. Atypical large mononuclear cells infiltrating among the fibers. Phosphotungstic acid-hematoxylin stain. From the same patient as Figure 75.  $\times 500$ .
- FIG. 79. Group III. Liver. Pericentral infiltration of large mononuclear elements. Atrophy of central ends of hepatic cell cords. From the same patient as Figure 75. A.I.P. neg. NM 162.  $\times 120$ .

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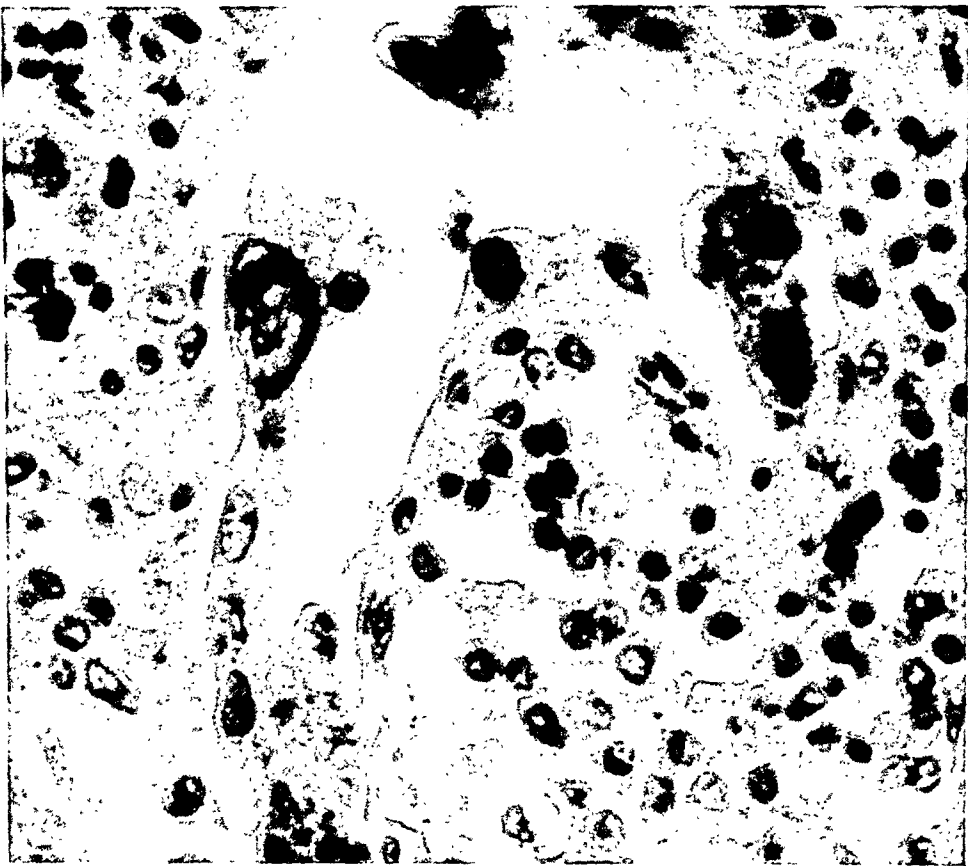
Pathology of Atomic Bomb Casualties

PLATE 144

FIG. 80. Group I. Ileum. Epithelial cell with enormous nucleus at base of crypt. Abundant plasmacytoid cells in the lamina propria. K-5. Yano. Male, 39 years of age. Approximately 1000 yds. Died on the sixth day. A.I.P. neg. HM 127.  $\times$  810.

FIG. 81. Group I. Intestine. Base of crypt. Slightly below and to the left of the center there is an epithelial cell in tripolar mitosis. K-2. Onishi. Male, 24 years of age. Approximately 800 yds. Died on the fourth day. A.I.P. neg. HM 125.  $\times$  650.

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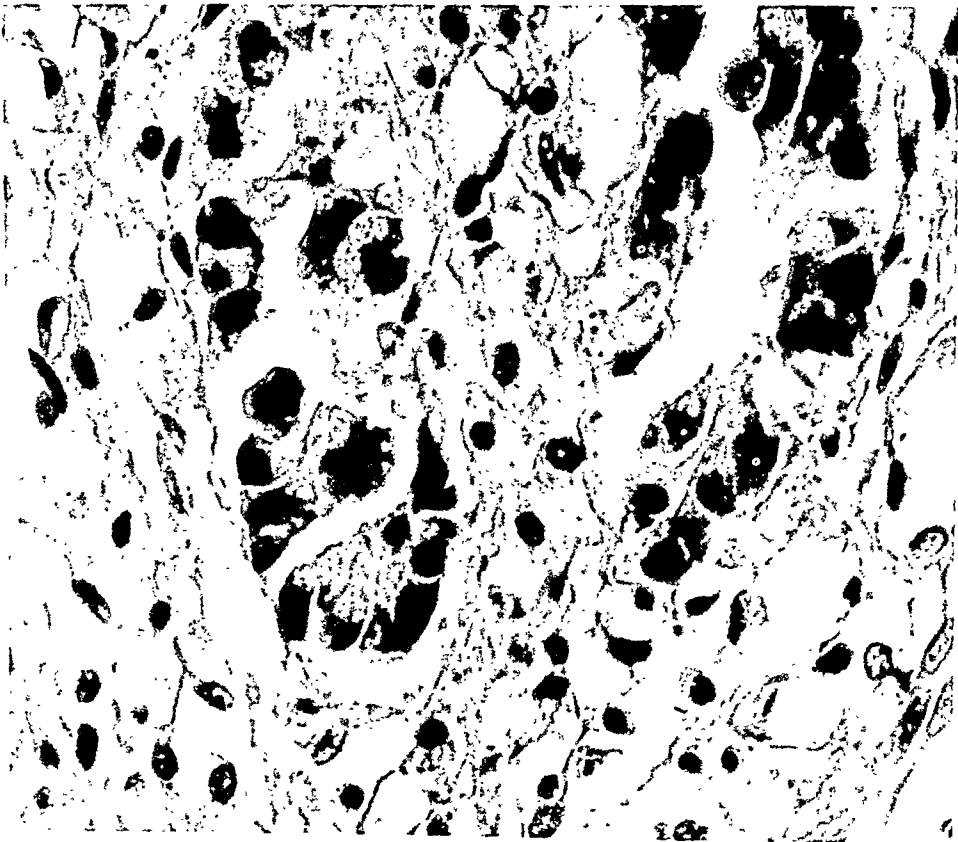


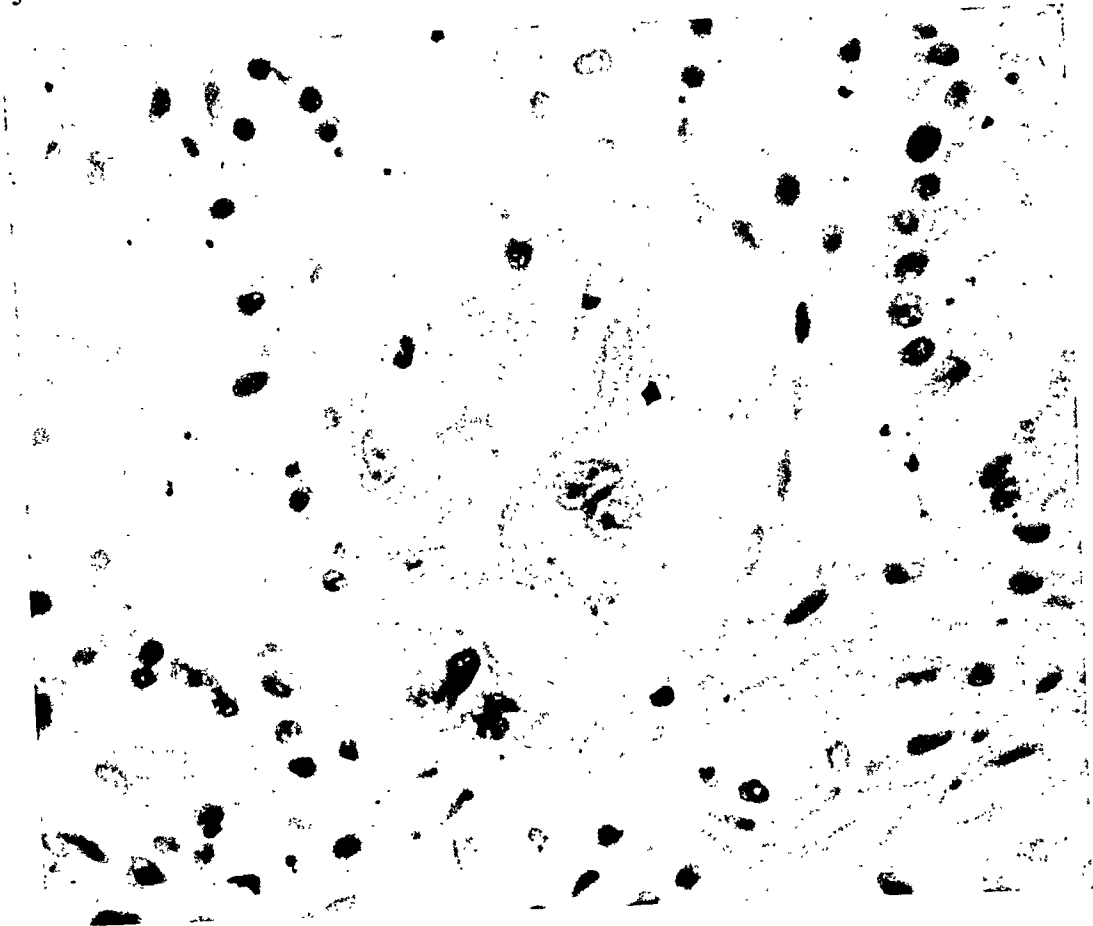


PLATE 145

FIG. 82. Group I. Intestine. Atypical epithelial cells at base of crypt. K-175. (Nagasaki.) Yamada. Female, 18 years of age. Distance unknown. Died on the eleventh day. A.I.P. neg. NM 159.  $\times 600$ .

FIG. 83. Group I. Intestine. Superficial ulcers containing masses of bacteria. Edema of submucosa. No leukocytic infiltration. From the same patient as Figure 82.  $\times 25$ .

82



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PLATE 146

FIG. 84. Group II. Stomach. Edema, hemorrhage, necrosis, and superficial ulcers of mucosa of stomach. The process is diffuse, except for a narrow band of well preserved mucous membrane along the lesser curvature and in a few places elsewhere. K-21. Iseoka. Male, 45 years of age. Approximately 1000 yds. Died on the 24th day. A.I.P. neg. HS 301.

FIG. 85. Group II. Ileocecal valve. Necrosis and hemorrhage of ileocecal valve and ascending colon. K-37. Takeda. Male, 25 years of age. Approximately 1000 yds. Died on the 29th day. A.I.P. neg. HS 320.

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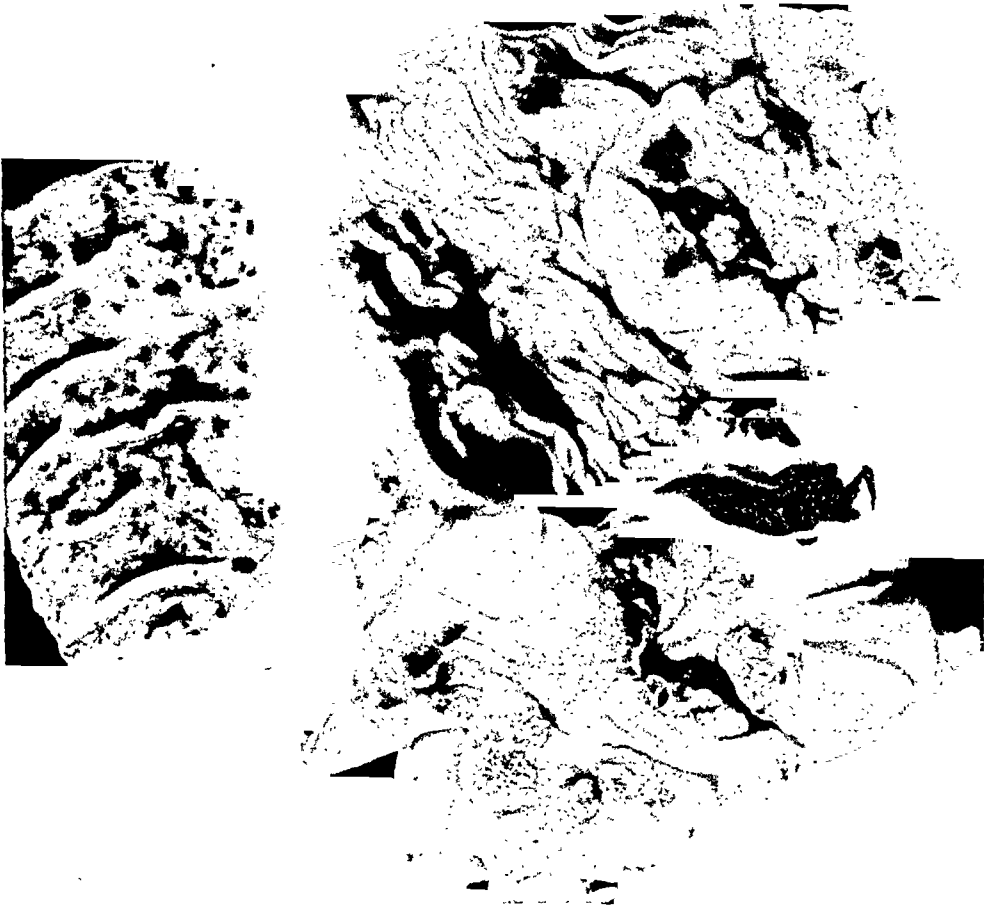
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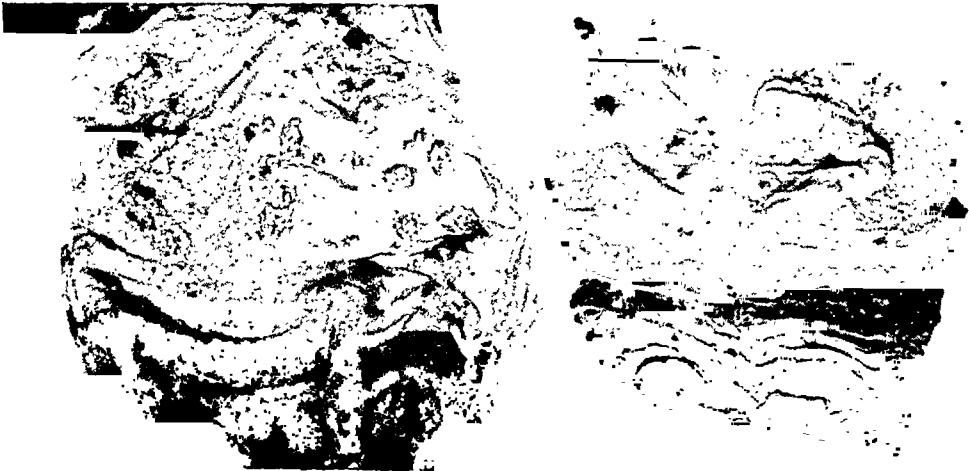
PLATE 147

- FIG. 86. Group II. Intestine. Focal hemorrhages of ileum. Hemorrhages and ulcers of ascending colon and ileocecal valve. K-35, Takahashi. Male, 31 years of age. Approximately 1000 yds. Died on the 28th day. A.I.P. neg. HS 317.
- FIG. 87. Intestine. Necrosis and hemorrhage of mucous membrane. Ileocecal valve involved. K-27. Omura. Male, 22 years of age. Approximately 1000 yds. Died on the 26th day. A.I.P. neg. HS 306.
- FIG. 88. Group II. Large intestine. Ulceration of mucous membrane. Fibrinous exudate adheres in large, irregular shreds. Diffuse hemorrhage of submucosa. K-38. Kamihara. Male, 22 years of age. Approximately 1000 yds. Died on the 29th day. A.I.P. neg. HS 321.

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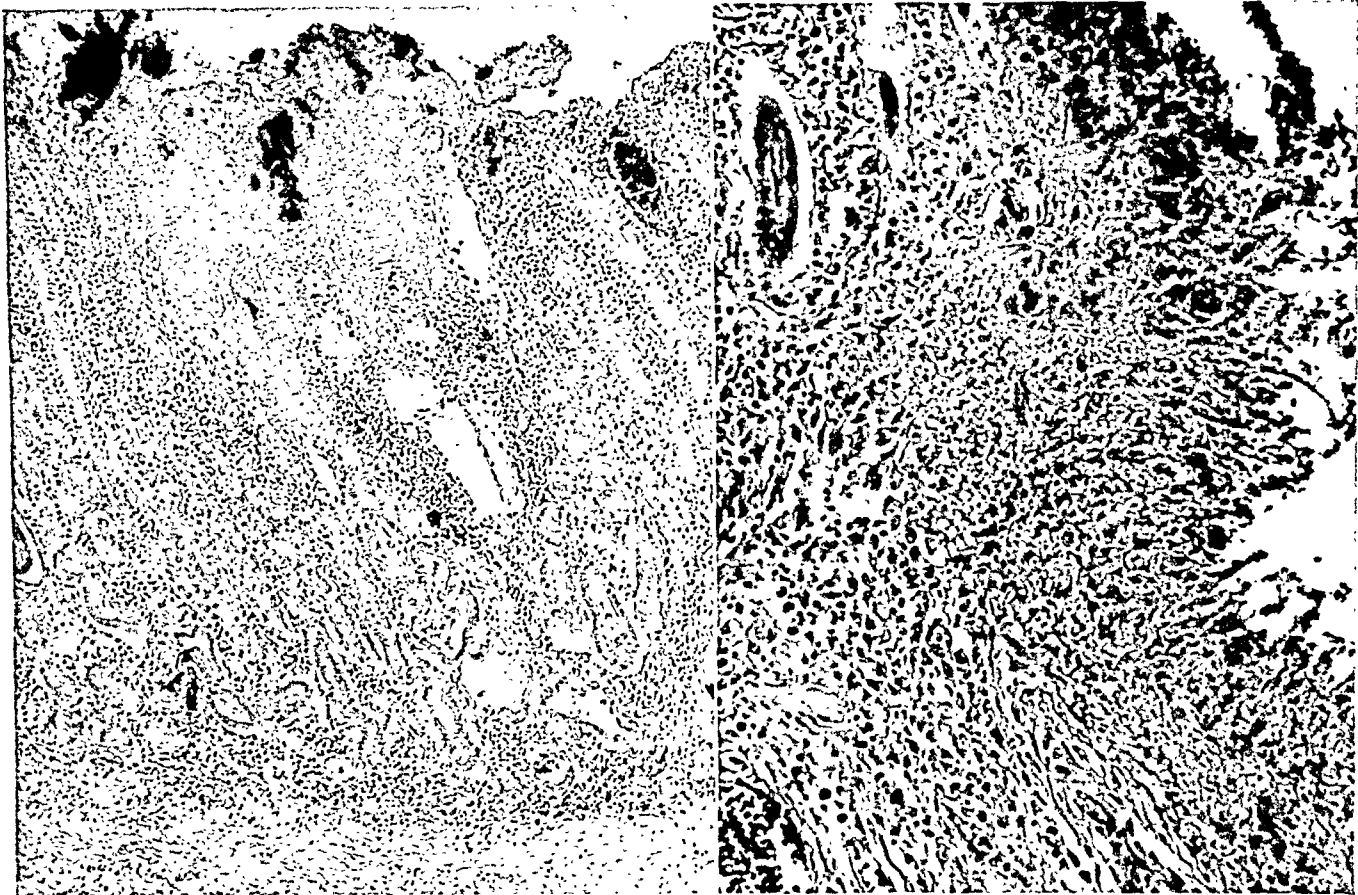


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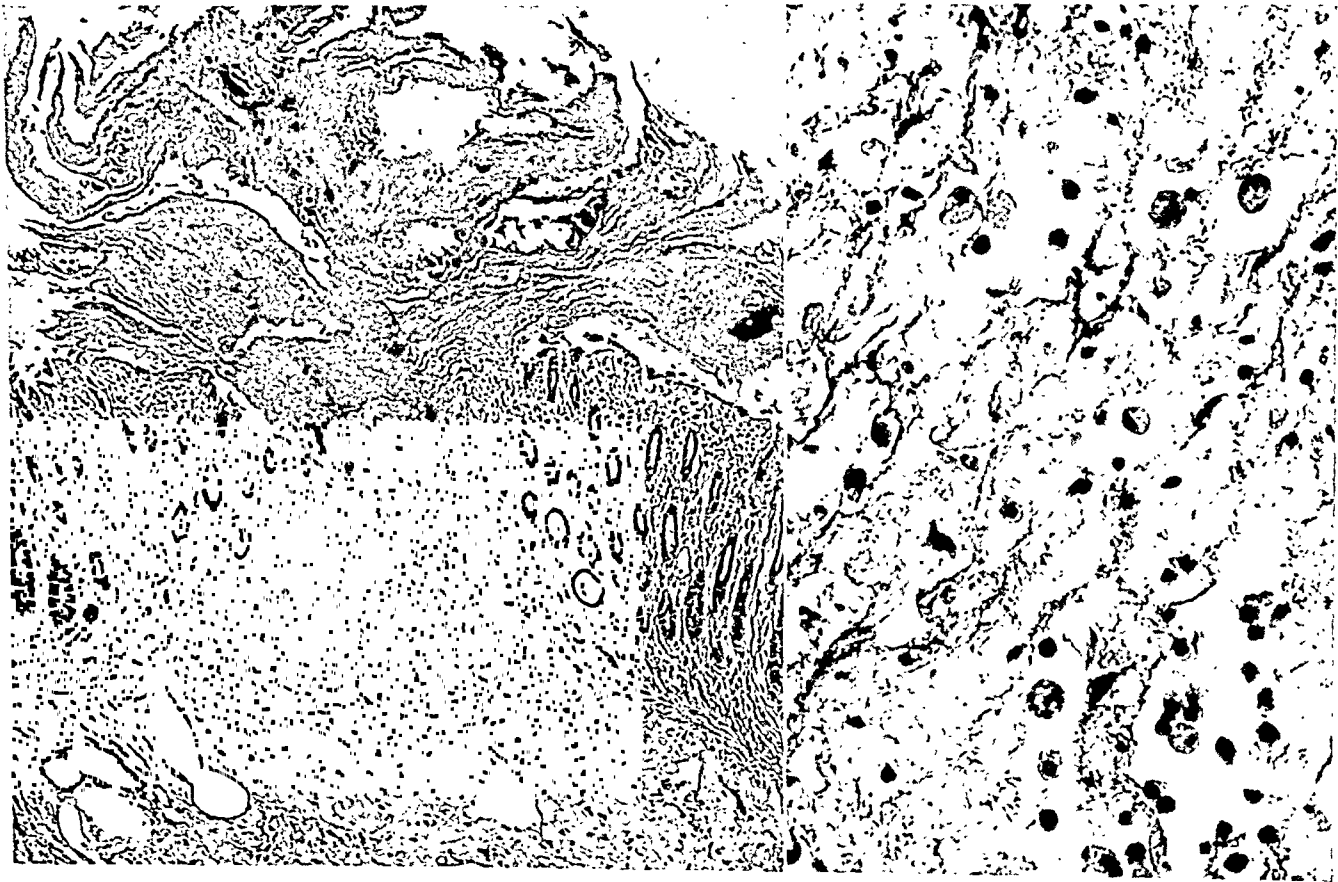
PLATE 148

- FIG. 89. Group II. Stomach. Focal necrosis. Bacterial masses at surface. Plasma cell infiltration of wall. From the same patient as Figure 84. A.I.P. neg. HM 146.  $\times 81$ .
- FIG. 90. Group II. Margin of ulcer. The exudate consists of small and large mononuclear cells and plasma cells, without polymorphonuclear leukocytes. K-43. Horinouchi. Male, 33 years of age. Approximately 1000 yds. Died on the 33rd day. A.I.P. neg. HM 235.  $\times 130$ .
- FIG. 91. Group II. Colon. Polypoid mass of necrotic tissue. Edema of mucosa and submucosa. No leukocytic infiltration.
- FIG. 92. Group III. Colon. Amebae in edematous areolar tissue of the submucosa. K-131. Takatani. Female, 39 years of age. Approximately 1500 yds. Died on the 46th day. A.I.P. neg. HM 339.  $\times 350$ .



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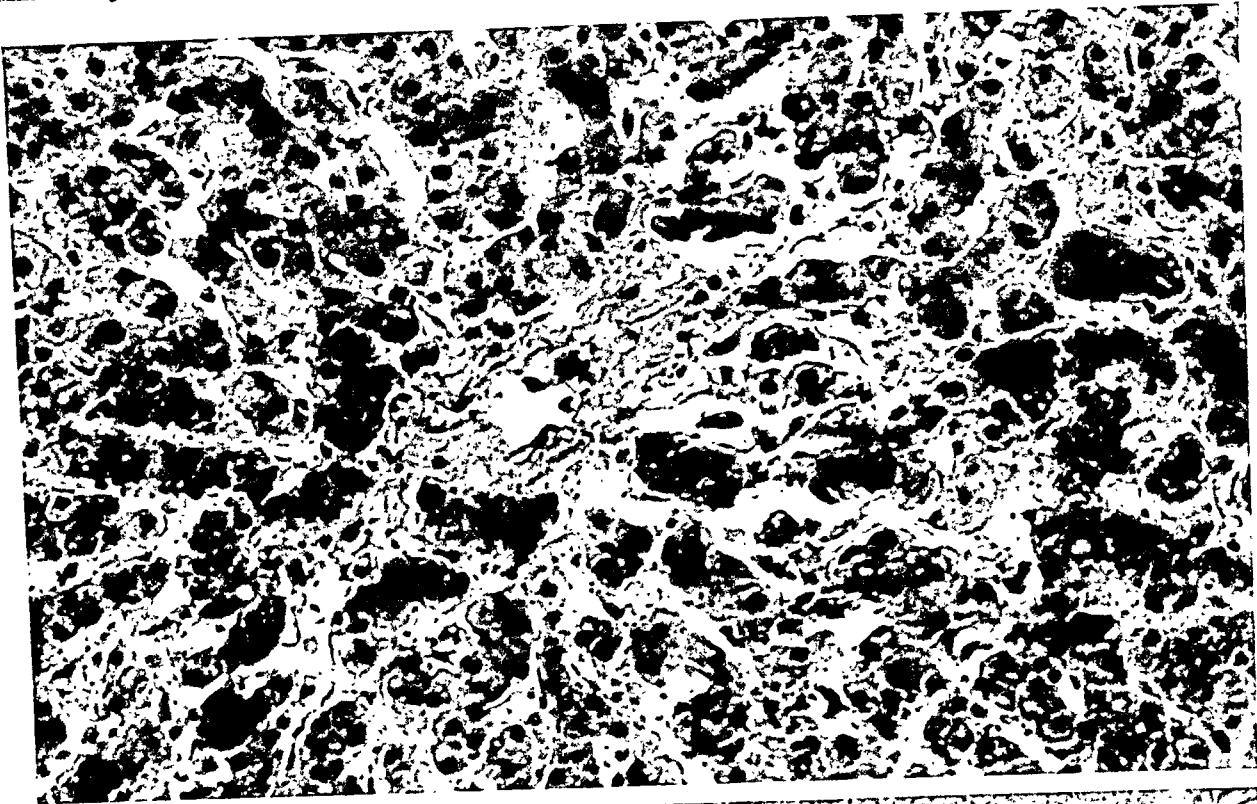
Pathology of Atomic Bomb Casualties



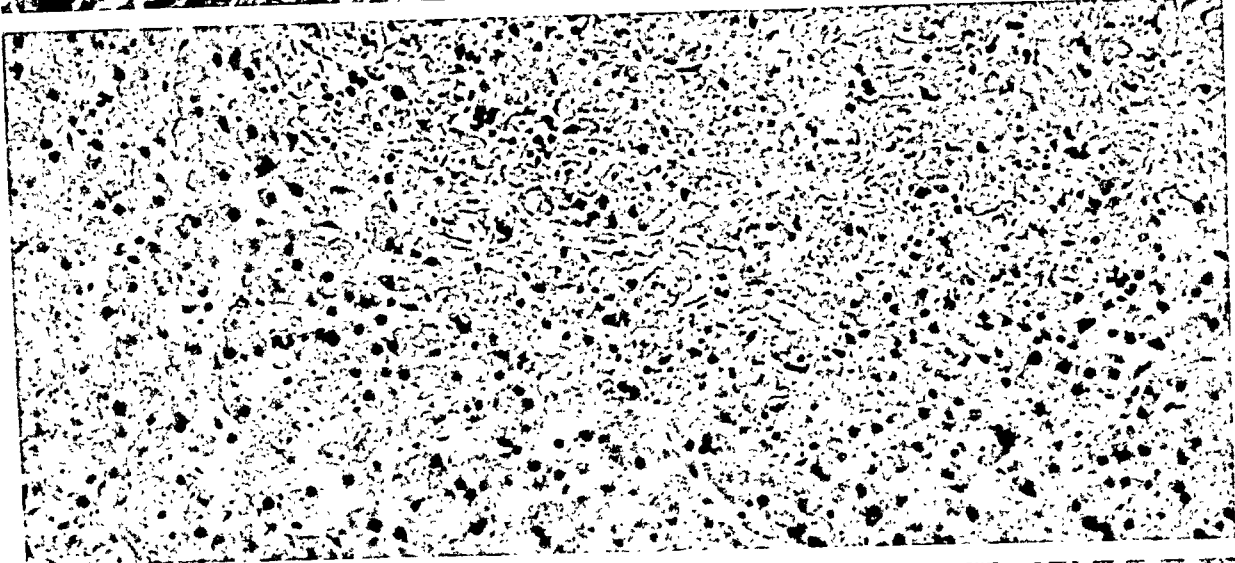
PLATE 149

- FIG. 93. Group I. Liver. Central congestion. Edema of connective tissue about the central vein. Large nuclei in the cells of the cords nearest the central venules. K-2. Onishi. Male, 24 years of age. Approximately 800 yds. Died on the fourth day.  $\times 250$ .
- FIG. 94. Group II. Liver. Focus of necrosis. The cellular exudate is scanty and consists almost entirely of plasma cells, many of which are represented only by granular pyknotic debris. K-89. Kuroki. Male, 23 years of age. Approximately 1000 yds. Died on the 21st day. A.I.P. neg. HM 273.  $\times 200$ .
- FIG. 95. Group III. Liver. Central necrosis; thickening of wall of central venule. Exudate of small and large mononuclear cells. K-14. Yamamoto. Male. 25 years of age. Approximately 1000 yds. Died on the 47th day. A.I.P. neg. HM 212.  $\times 115$ .

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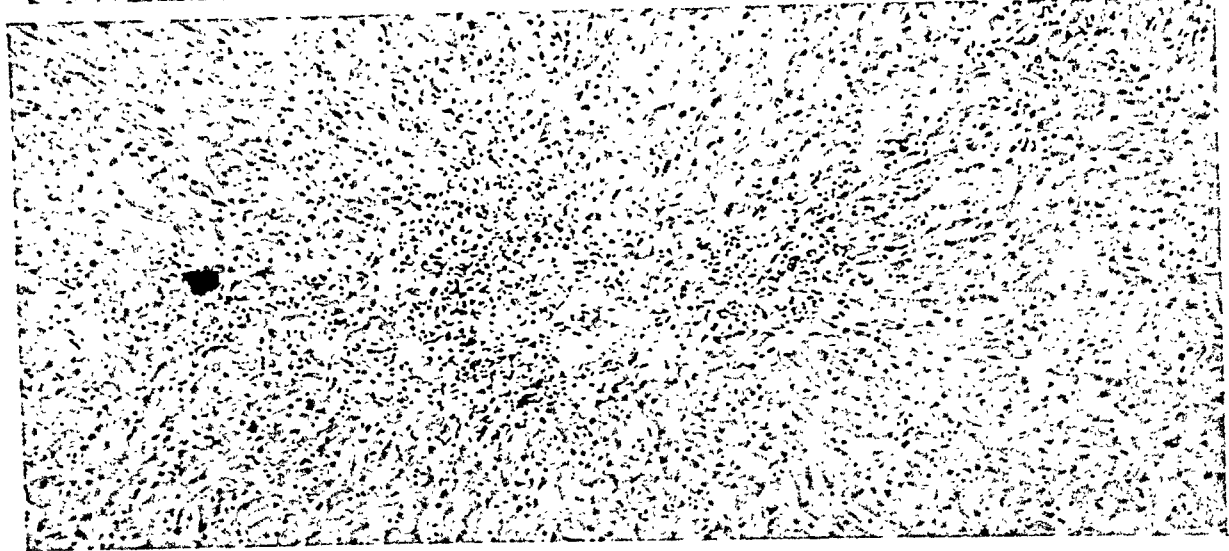
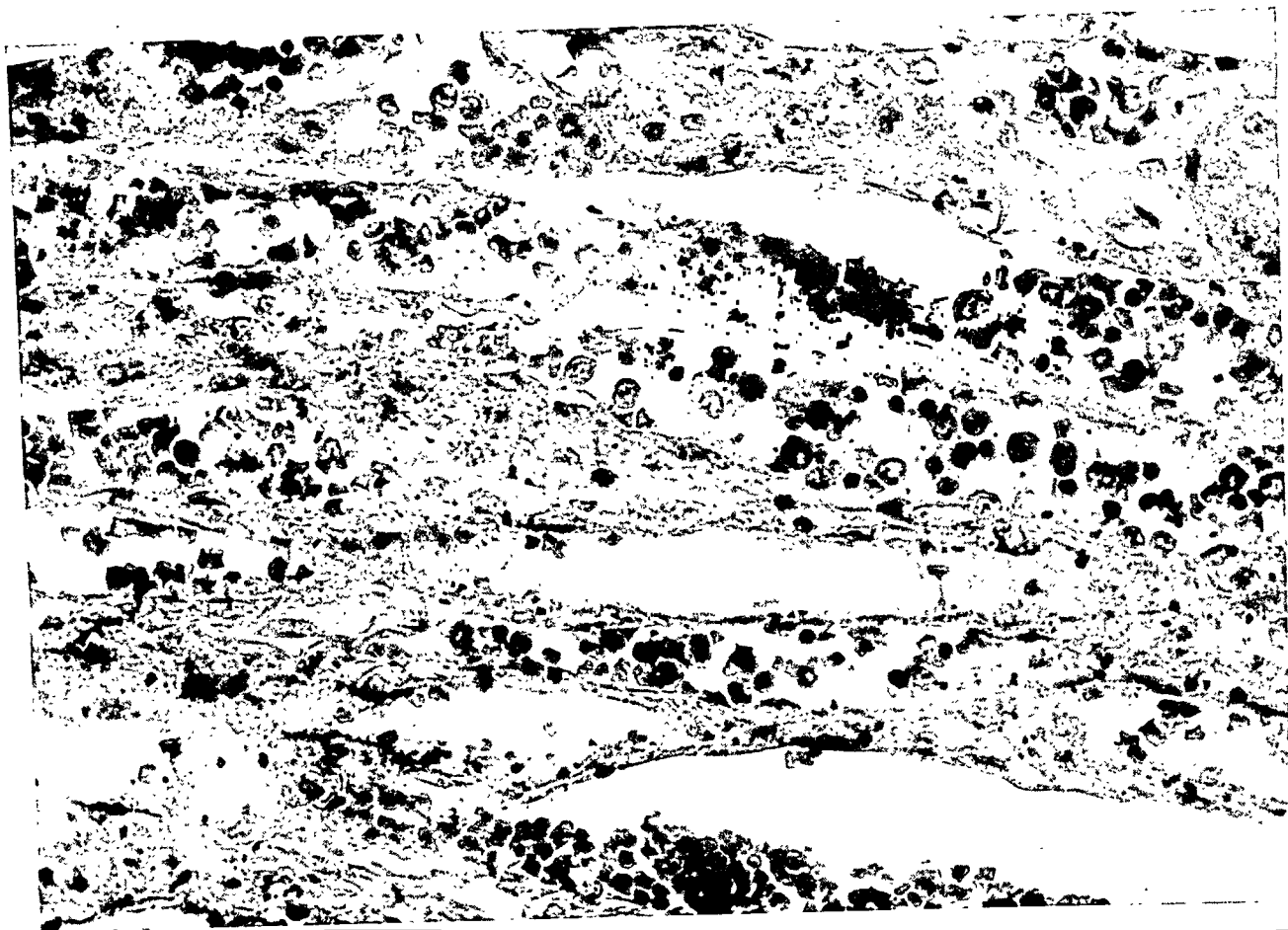


PLATE 150

FIG. 96. Group I. Kidney. Sinusoids at corticomedullary junction containing large mononuclear cells, one in mitosis. K-9. Sakamoto. Male, 25 years of age. Approximately 1500 yds. Died on the eighth day. A.I.P. neg. HM 137.  $\times 400$ .

FIG. 97. Group II. Kidney. Petechiae in parenchyma of kidney, hemorrhage in mucous membrane of pelvis. There was no evidence of glomerulonephritis histologically. K-30. Nagashima. Male, 23 or 28 years of age (variously stated). Approximately 1000 yds. Died on the 26th day. A.I.P. neg. HS 312.

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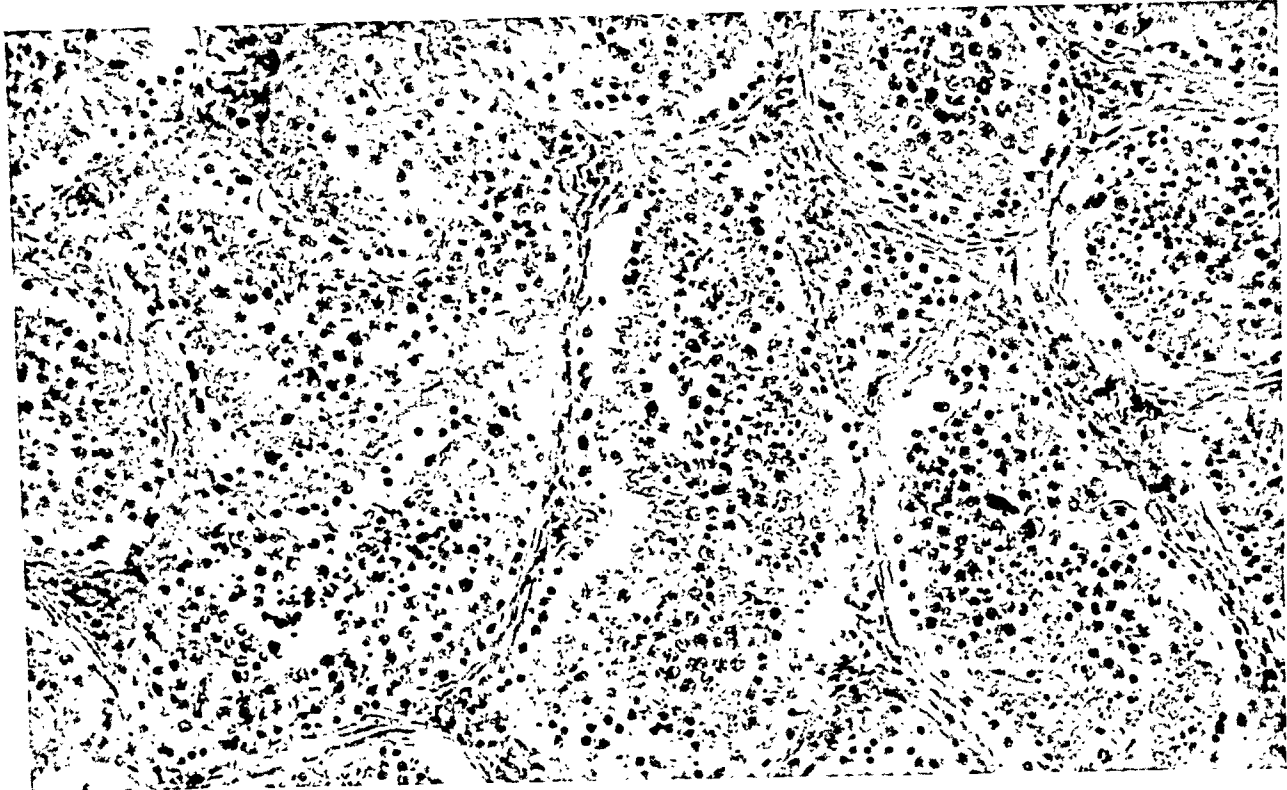
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Pathology of Atomic Bomb Casualties

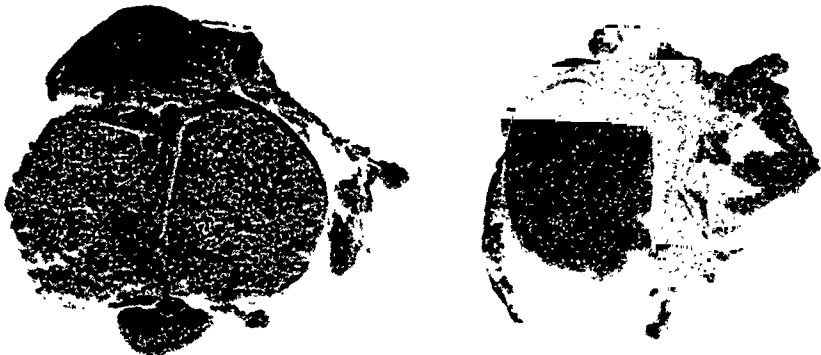
PLATE 151

- FIG. 98. Group I. Testis. Sloughing of spermatogonia from basement membrane whereon rest Sertoli cells in increased numbers. Relatively few spermatids and mature spermatozoa in lumina of tubules. Mitotic figures are relatively rare. K-2. Onishi, 24 years of age. Approximately 800 yds. Died on the fourth day. A.I.P. neg. HM 122.  $\times 160$ .
- FIG. 99. Group II. Testes. Atrophy. K-30. Nagashima, 23 or 28 years of age (variously stated). Died on the 26th day. A.I.P. neg. HS 313.
- FIG. 100. Group II. Testis. Atrophy. Necrotic remnants of germinal epithelium and its derivatives in lumina of tubules. Edema of interstitial tissues. Hyaline sub-endothelial changes of arterioles. K-28. Kawaura, 23 years of age. Approximately 1000 yds. Died on the 26th day. A.I.P. neg. HM 150.  $\times 125$ .

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PLATE 152

- FIG. 101. Group II. Testis. Atrophy of germinal epithelium and its derivatives. Hyperplasia of interstitial tissue. K-39. Takeuchi, 29 years of age. Approximately 1000 yds. Died on the 29th day. A.I.P. neg. HM 228.  $\times 115$ .
- FIG. 102. Group II. Testis. Atrophy of tubules. Near the basement membrane, among the Sertoli cells, is an ovoid cell with a hyperchromatic nucleus, considered to be a persistent element of the germinal epithelium. K-46. Kurihara, 22 years of age. Approximately 800 yds. Died on the 33rd day. A.I.P. neg. HM 241.  $\times 450$ .
- FIG. 103. Group II. Testis. Atrophy. Tubule containing "giant cell" apparently produced by fusion and compaction of the cytoplasm of spermatids, some of which are still isolated. K-40. Motoyama, 29 years of age. Approximately 1000 yds. Died on the 30th day. A.I.P. neg. HM 231.  $\times 450$ .
- FIG. 104. Group II. Testis. Atrophy. "Giant cell" in lumen of tubule, which is lined by Sertoli cells. The "giant" cell probably has resulted from compaction and fusion of spermatids. K-91. Kato, 24 years of age. Approximately 1000 yds. Died on the 30th day. A.I.P. neg. HM 274.  $\times 400$ .

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Pathology of Atomic Bomb Casualties



PLATE 153

FIG. 105. Group III. Testis. Cessation of spermatogenesis. The tubules, whose basement membranes have not become thickened, are lined exclusively by Sertoli cells. The interstitial tissue is not hyperplastic. K-50. Kijima, 31 years of age. Approximately 1000 yds. Died on the 100th day. A.I.P. neg. HM 143.  $\times 115$ .

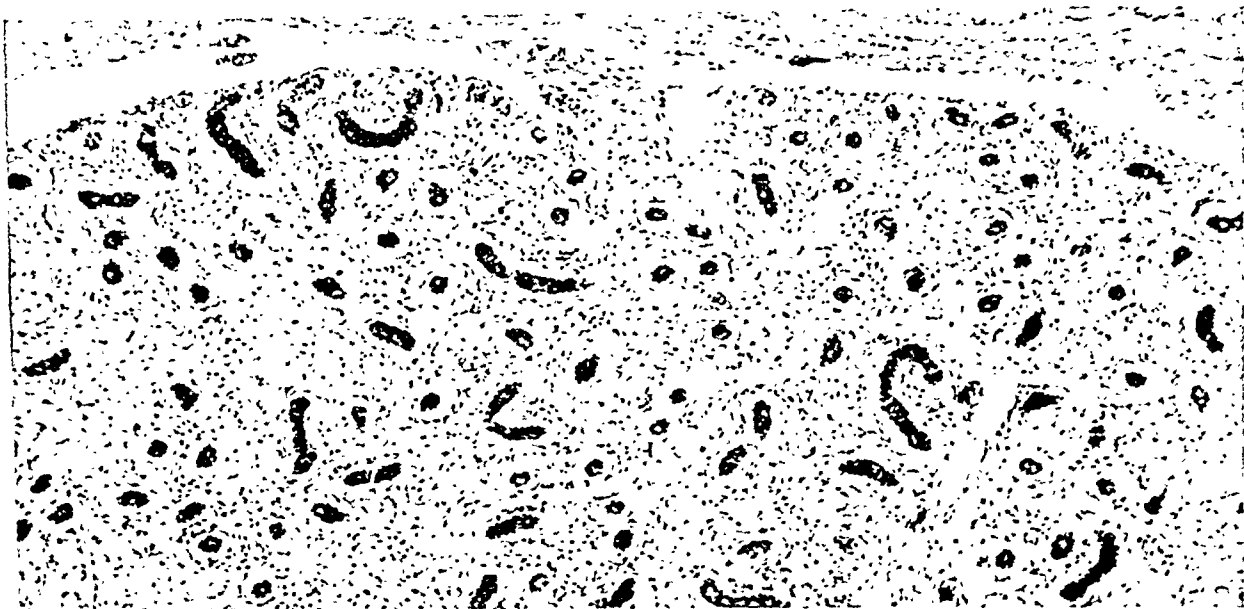
FIG. 106. Group III. Immature testis. Thickening of basement membranes of tubules. The epithelium appears slightly shrunken but otherwise well preserved. K-128. Fukuhara, 8 years of age. Between 500 and 1000 yds. Died on the 40th day. A.I.P. neg. HM 338.  $\times 100$ .

FIG. 107. Group III. Prostate. Atrophy. Grossly, this prostate was approximately one-half the usual size. From the same patient as Figure 105. A.I.P. neg. HM 144.  $\times 95$ .

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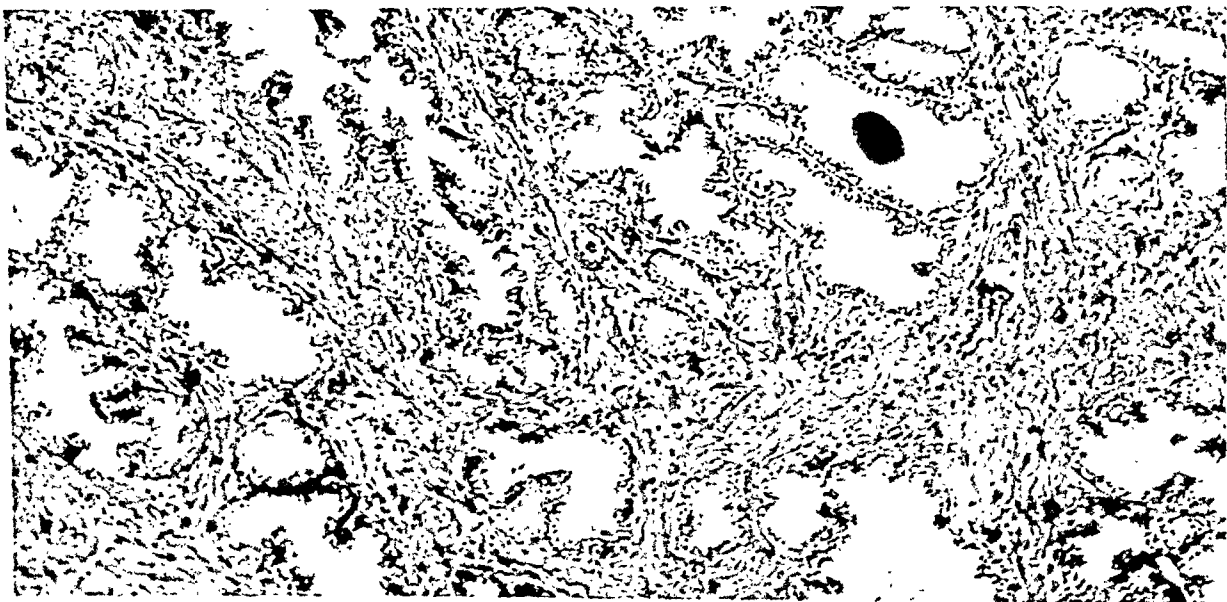


PLATE 154

FIG. 108. Group II. Pelvic organs. Hemorrhages in endometrium and in right ovary. K-36. Morita, 21 years of age. Approximately 1000 yds. Died on the 28th day. A.I.P. neg. HS 318.

FIG. 109. Group II. Ovary. Paucity of primary follicles. Atresia of surviving follicles. No developing follicles. Corpora albicantia present elsewhere in this ovary. K-119. Nagado, 26 years of age. Approximately 1300 yds. Died on the 23rd day. A.I.P. neg. HM 303.  $\times 115$ .

108



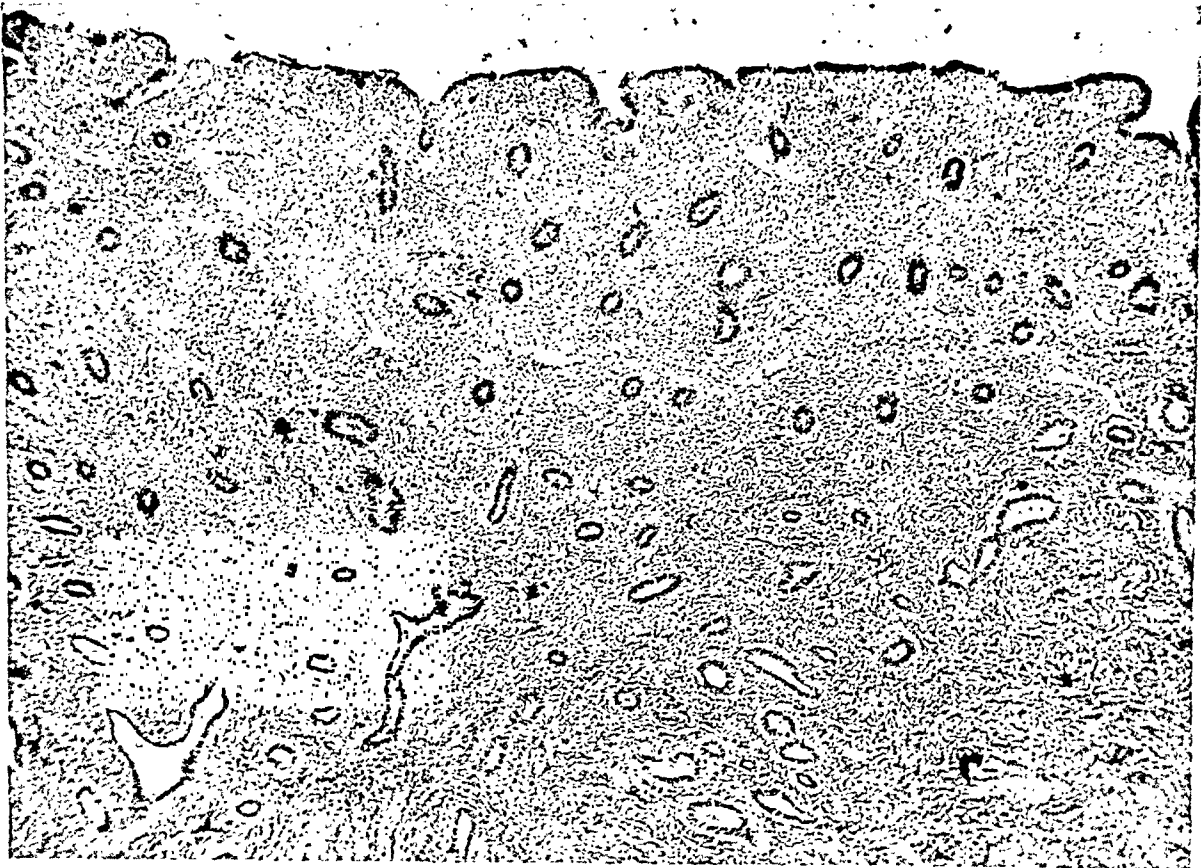
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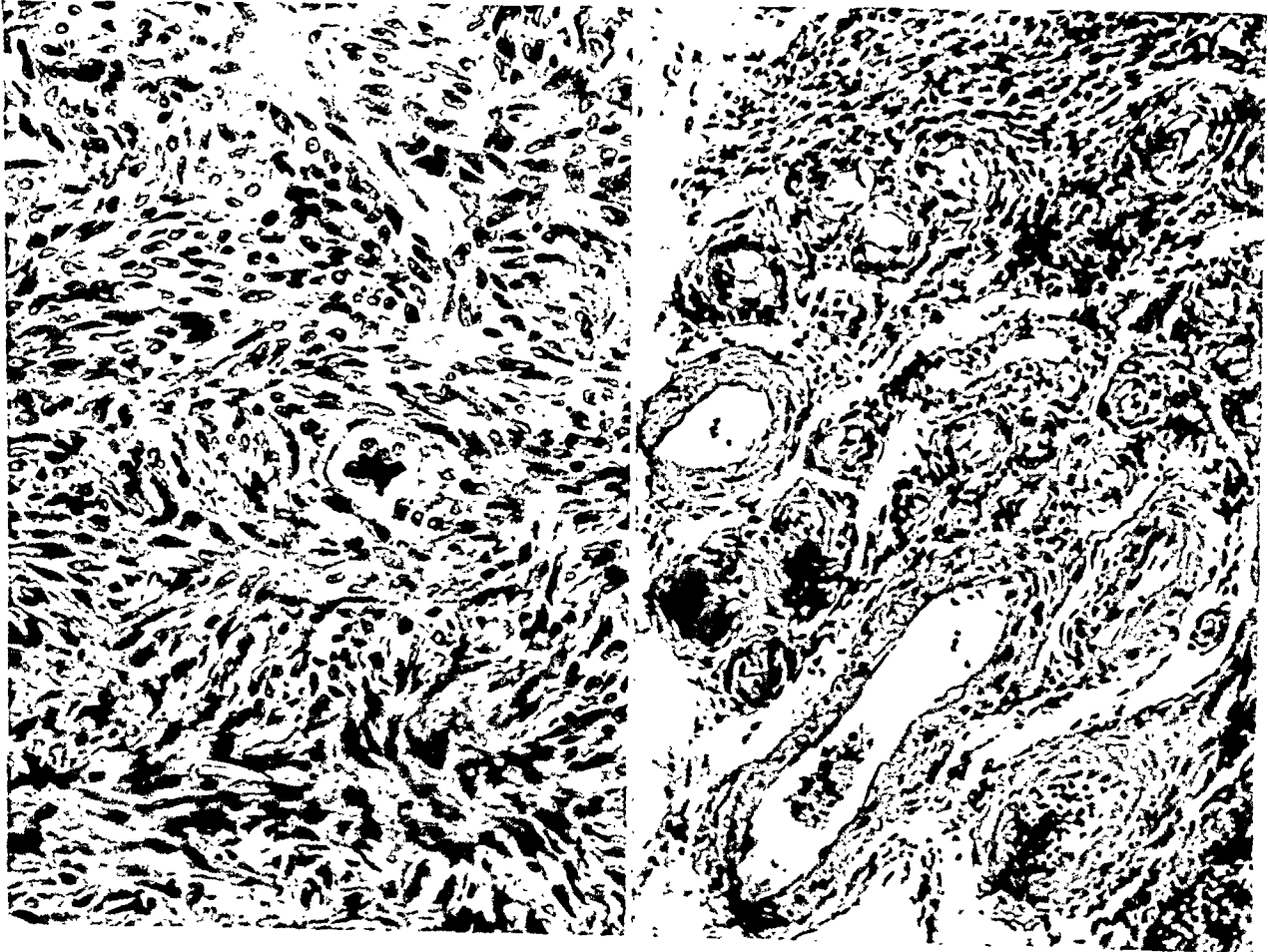
PLATE 155

- FIG. 110. Group II. Endometrium in "resting phase." K-47. Naka, 35 years of age. Approximately 800 yds. Died on the 18th day. A.I.P. neg. HM 245.  $\times 50$ .
- FIG. 111. Group II. Ovary. Atretic follicle. Granulosa cells clumped at center. Enlargement of a portion of Figure 109.  $\times 450$ .
- FIG. 112. Group II. Ovary. Deposits of hyaline refractile acidophilic material beneath endothelium of blood vessels. Similar to changes seen in stroma of testis. From the same patient as Figure 109. A.I.P. neg. HM 30.  $\times 175$ .

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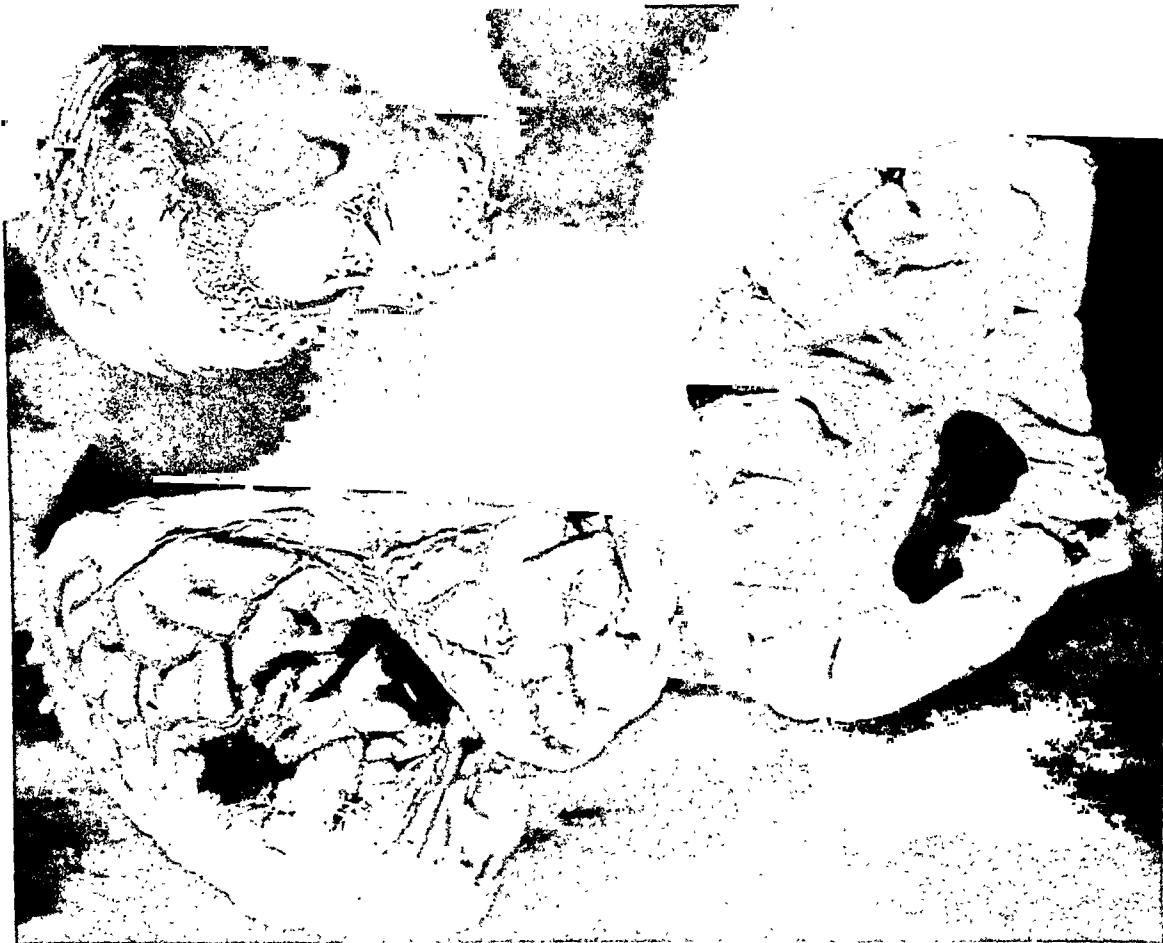
Pathology of Atomic Bomb Casualties

PLATE 156

FIG. 113. Group II. Sub-pial hemorrhage of frontal lobe. Massive hemorrhage in medullary cistern of sub-arachnoid space. Petechiae of sub-ependyma of lateral ventricle. K-42. Moriseko. Male, 27 years of age. Approximately 1000 yds. Died on the 31st day. A.I.P. neg. HS 324.

FIG. 114. Group II. Brain. Focus of necrosis in medulla, surrounded by hemorrhage. A small bacterial mass which appears black in the photomicrograph lies near the center of the lesion. K-60. Shigeta. Female, 44 years of age. Approximately 800 yds. Died on the 36th day. A.I.P. neg. HM 333.  $\times 100$ .

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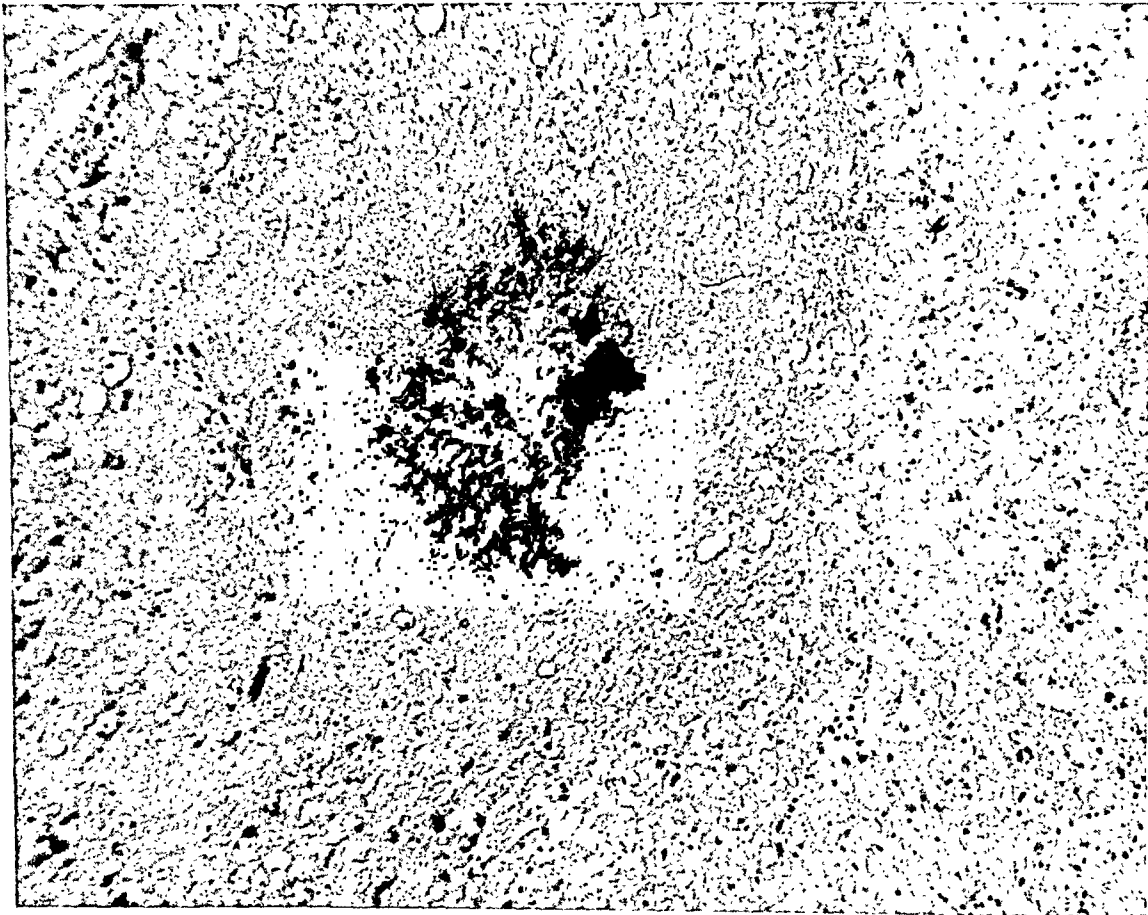
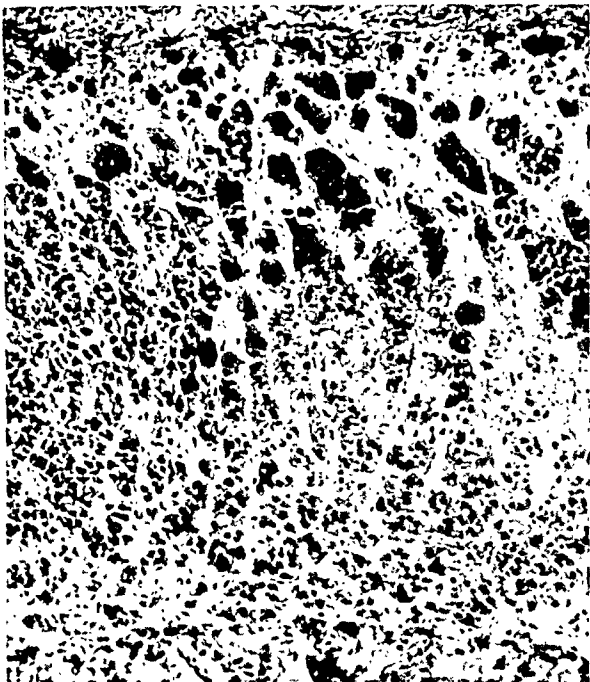




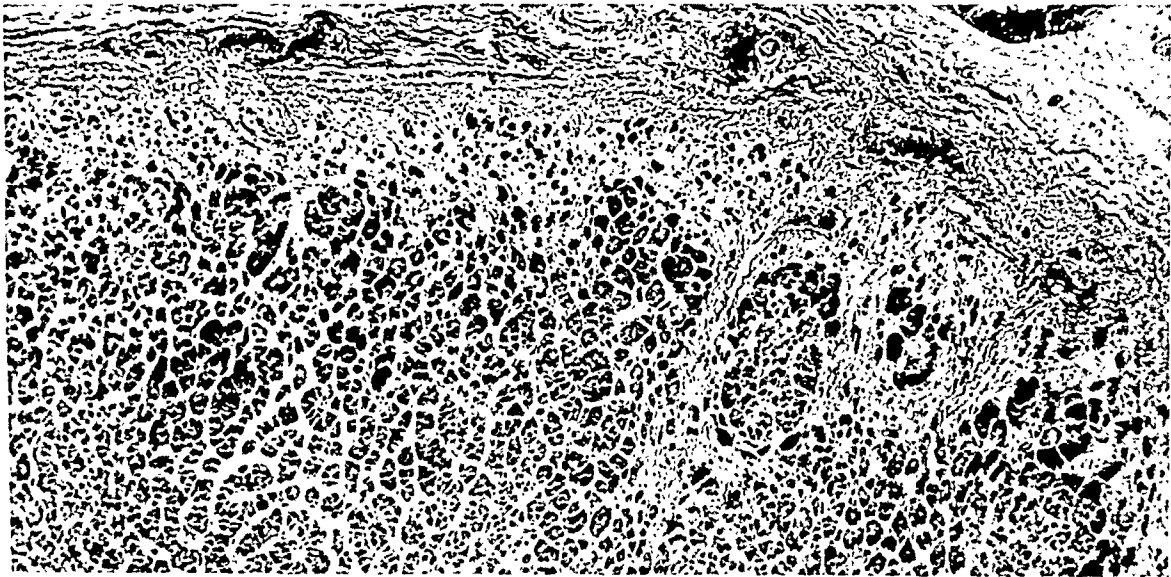
PLATE 157

- FIG. 115. Group III. Adrenal. Focal atrophy with decrease in thickness of cortex. Focal fatty changes in cortex. K-96. Sakoda. Male, 33 years of age. Approximately 1000 yds. Died on the 97th day.  $\times 130$ .
- FIG. 116. Group II. Atrophy of zona glomerulosa. Loss of lipid. Edema of subcapsular connective tissue. Masson's stain. K-2. Onishi. Male, 24 years of age. Approximately 800 yds. Died on the fourth day. A.I.P. neg. HM 124.  $\times 60$ .
- FIG. 117. Group II. Adrenal. Focus of necrosis in fascicular stratum of cortex. Infiltration of large fat-filled phagocytes. K-45. Akagi. Male, 28 years of age. Approximately 1000 yds. Died on the 33rd day. A.I.P. neg. HM 236.  $\times 265$ .
- FIG. 118. Group II. Adrenal. Fatty changes of scattered cells and groups of cells in the cortex. Several epithelial cells in the fascicular stratum are in mitosis. From the same patient as Figure 117. A.I.P. neg. HM 237.  $\times 210$ .

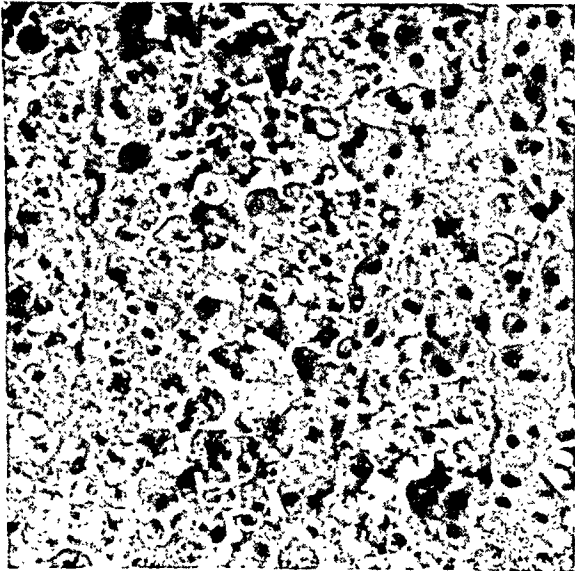
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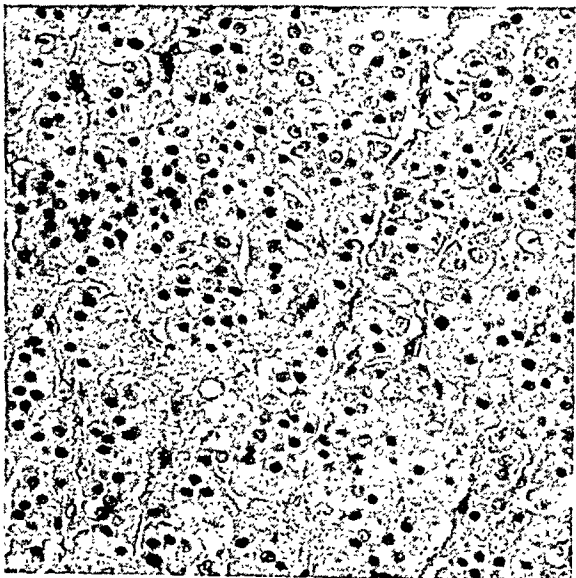


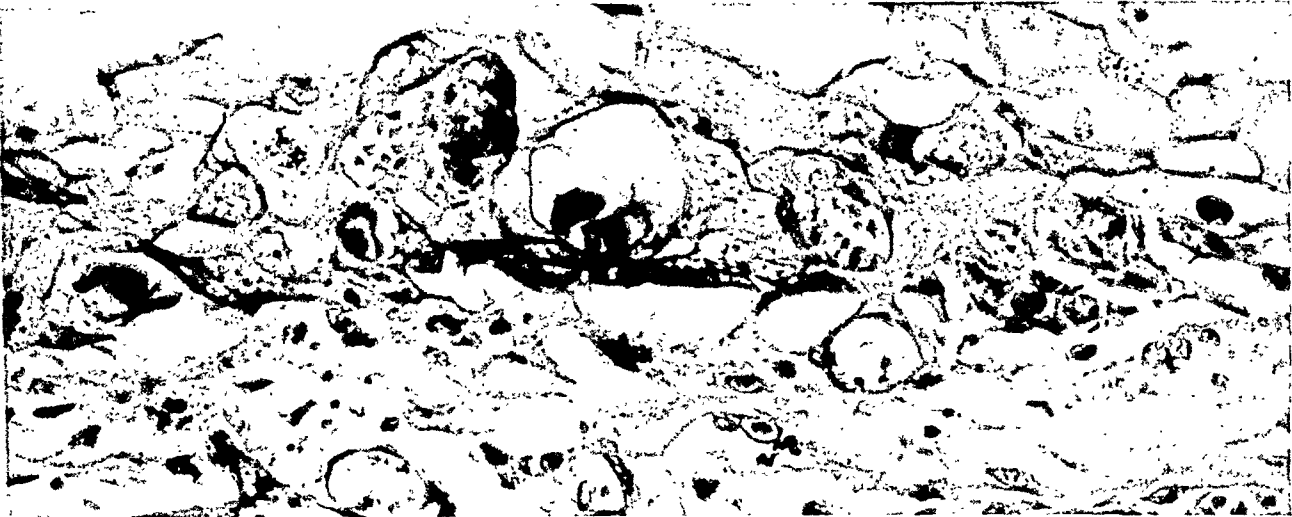
PLATE 158

- FIG. 119. Group I. Wall of pharynx. Swelling, vacuolation, fragmentation, and desquamation of the squamous epithelium. Edema of connective tissue. Atrophy of lymphoid tissue. Tremendous lymphectasia. Occasional plasma cells, mast cells, and large mononuclear elements scattered throughout the areolar tissue. K-98. Tamai. Male, 19 years of age. Distance unknown. Died on the tenth day.  $\times 100$ .
- FIG. 120. Group I. Pharynx. Epithelial swelling and fragmentation. Edema of deeper layers of wall. Enlargement of a portion of Figure 119. A.I.P. neg. HM 332.  $\times 450$ .
- FIG. 121. Group I. Swelling and vacuolation of some squamous epithelial cells. Atrophy of others. Swelling and loss of staining qualities of nuclei. Micro-nuclei (?) in one cell near surface. Parakeratosis at surface. Dilatation of lymphatics and edema of tongue. From the same patient as Figure 119. A.I.P. neg. HM 330a.  $\times 130$ .

119



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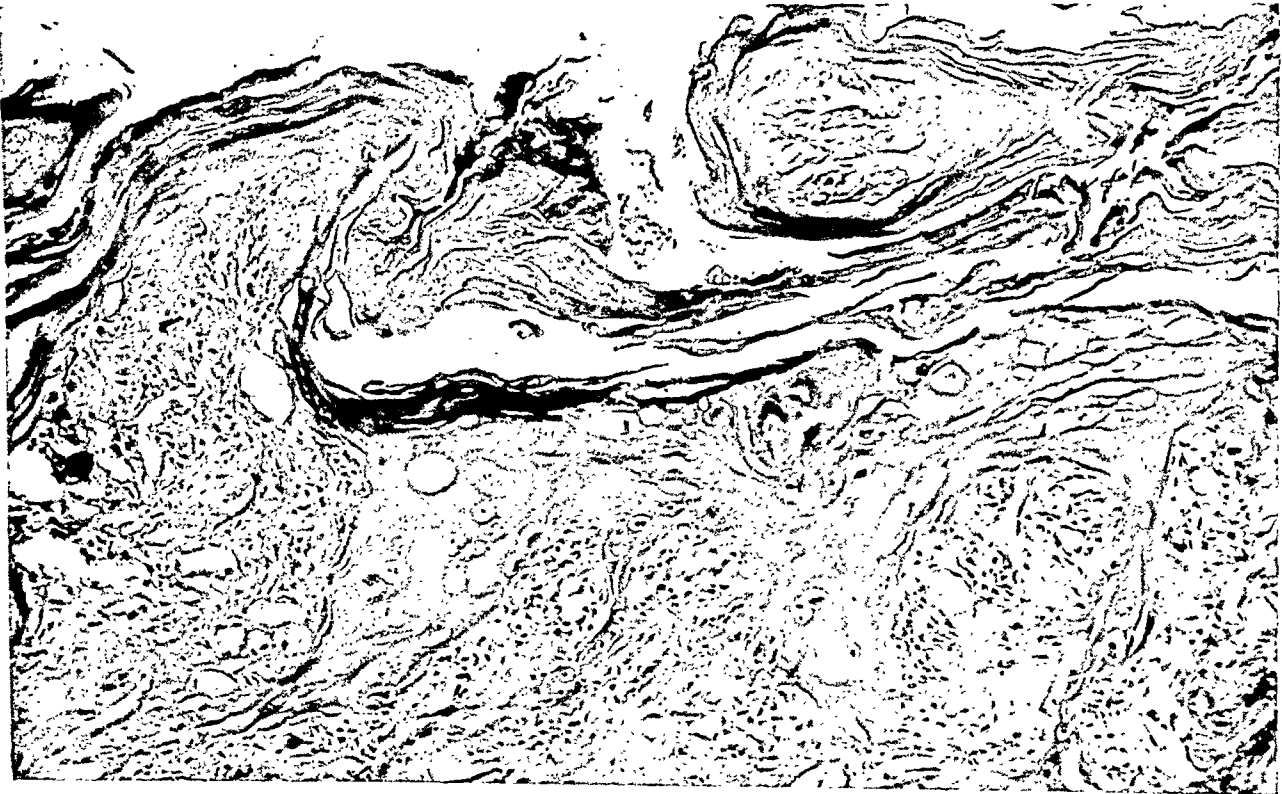


PLATE 159

- FIG. 122. Group II. Neck organs. Necrosis of lining membrane of epiglottis, larynx, and trachea. Necrosis and enlargement of left tonsil. Foci of necrosis in the right tonsil, which is somewhat smaller. K-21. Iseoka. Male, 45 years of age. Approximately 1000 yds. Died on the 24th day. A.I.P. neg. HS 300.
- FIG. 123. Group II. Neck organs. Necrosis and hemorrhage of faucial and lingual tonsils, pyriform sinus, and epiglottis. K-44. Araki. Male, 22 years of age. Approximately 1000 yds. Died on the 33rd day. A.I.P. neg. HS 328.
- FIG. 124. Group II. Tongue. Necrosis and hemorrhage. K-29. Murakami. Male, 22 or 24 years of age (variously stated). Approximately 1000 yds. Died on the 26th day. A.I.P. neg. HS 310.
- FIG. 125. Group II. Tonsil. Necrosis. Absence of polymorphonuclear leukocytic barrier between the necrotic and non-necrotic tissue. K-47. Naka. Female. 35 years of age. Approximately 800 yds. Died on the 18th day. A.I.P. neg. HM 264.  $\times 130$ .



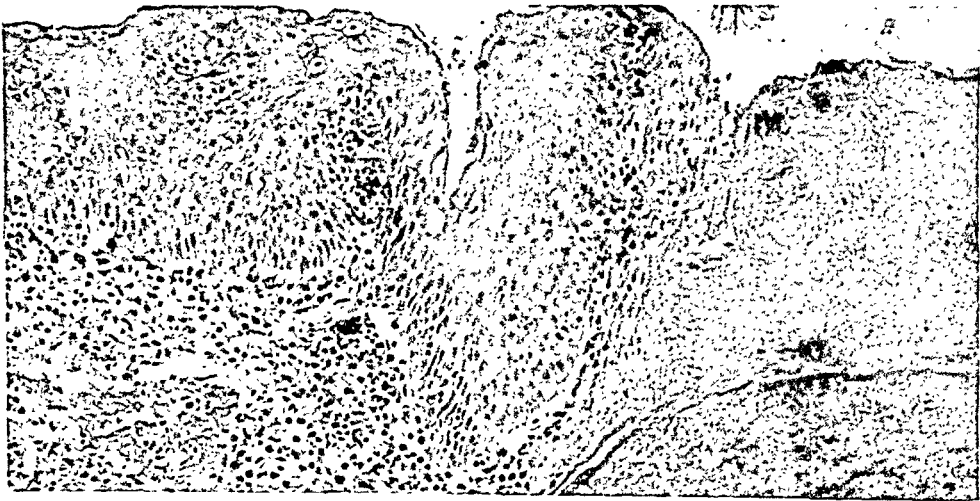
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PLATE 160

FIG. 126. Group II. Epiglottis. Necrosis and infiltration of small mononuclear and plasma cells. Occasional polymorphonuclear leukocytes. Cartilage intact. K-56. Kawamura. Female, 51 years of age. Approximately 1100 yds. Died on the 36th day. A.I.P. neg. HM 270.  $\times 115$ .

FIG. 127. Group II. Pharynx. Thrush. Mycelium (probably of monilia). From the same patient as Figure 126. A.I.P. neg. HM 271.  $\times 130$ .

126



127





# PLATE 161

FIG. 128. Group II. Patient with petechiae of skin. Epilation appeared on August 18, gingival hemorrhages and petechiae of skin began on August 29. Step-like rise of temperature began on August 31. Necrotizing tonsillitis was noted on September 1, delirium on September 2, and death occurred on September 3, 1945. Laboratory data on day of death: red blood cells, 2.03 millions; hemoglobin, 40 per cent; white blood cells, 1900; platelets, 10,400; bleeding time, 46 minutes. Hashimoto. Male, 21 years of age. Exact distance unknown. No record of autopsy. Photograph made by Japanese medical officers of Tokyo 1st Military Hospital 2 hours before the patient expired. A.I.P. neg. HP 135.

FIG. 129. Group II. Skin. Low-power view of margin of ulcer, and adjacent tissues. K-47. Naka. Female, 35 years of age. Approximately 800 yds. Died on the 18th day. A.I.P. neg. HM 344.  $\times 30$ .

FIG. 130. Group II. Skin. Ulcer. Bacterial masses in necrotic material. Plasma cells, large mononuclear cells and extravasated erythrocytes at base. K-29. Murakami. Male, 24 or 22 years of age (variously stated). Approximately 1000 yds. Died on the 26th day. A.I.P. neg. HM 296.  $\times 115$ .

FIG. 131. Group II. Skin. Margin of ulcer. Necrosis of outer layers of epithelium. Pigment remains in basal cells. Congestion of blood vessels and hemorrhages. Bacteria in necrotic material at surface of ulcer. No leukocytic infiltration. Enlargement of a portion of Figure 129. A.I.P. neg. HM 299.  $\times 115$ .

FIG. 132. Group II. Skin, at distance from ulcer. Vacuolation of epithelial cells and shrinkage of nuclei. Irregularity of distribution of pigment and absence of mitotic figures in basal layer. Small mononuclear cells about dilated blood vessels in corium. Enlargement of a portion of Figure 129. A.I.P. neg. HM 345.  $\times 150$ .

128



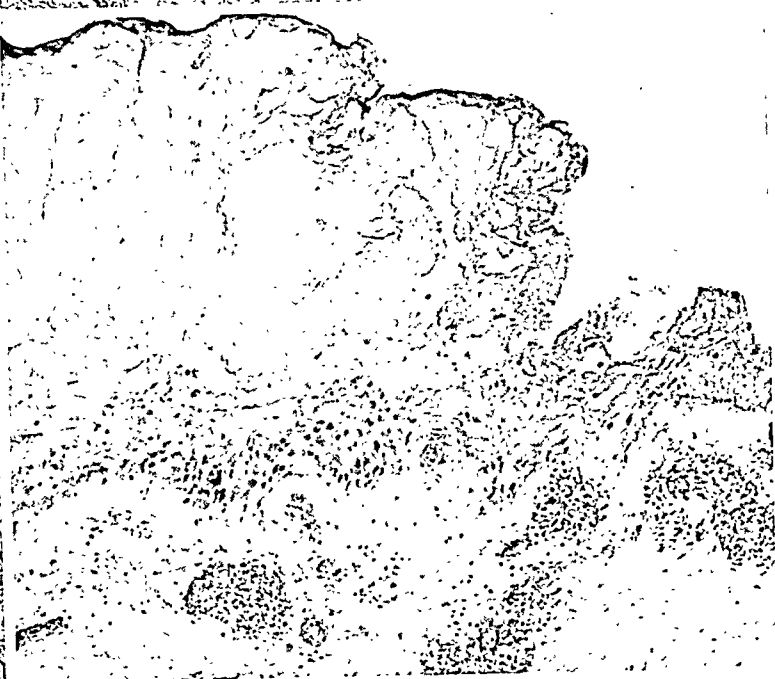
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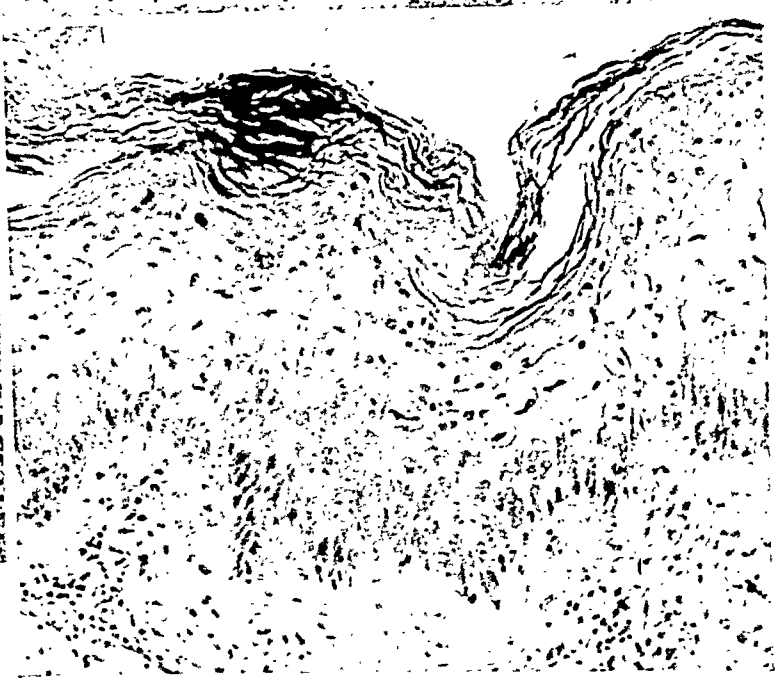
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# PLATE 162

- FIG. 133. Regrowth of hair, following epilation. Patient had been approximately 1000 yds. from the explosion on the upper floor of a two-story Japanese building in Hiroshima. Epilation began on August 20, 2 weeks after the bombing. His white blood cell count had fallen to as low as 900 on September 4 and he had had fever, petechiae, and ulcerative and hemorrhagic gingivitis from which he recovered. Approximately 2½ months after the bombing. A.I.P. neg. HP 125.
- FIG. 134. Group III. Scalp. Regenerating hair follicle to illustrate typical structure. Occasional mitotic figures among matrix cells. Well defined Henle's and Huxley's layers and external root sheath. Cuticle is forming. K-14. Yamamoto. Male, 25 years of age. Approximately 1000 yds. Died on the 47th day. A.I.P. neg. HM 210.  $\times 225$ .
- FIG. 135. Group II. Scalp. Atrophy of hair follicle, thickening of glassy and basement membranes. Failure of differentiation of internal root sheath. Irregularity of distribution of pigment. Vacuoles between epithelium and glassy membrane. K-45. Akagi. Male, 38 years of age. Approximately 1000 yds. Died on the 33rd day. A.I.P. neg. HM 238.  $\times 130$ .
- FIG. 136. Group II. Scalp. Hair follicle. Atrophy. Failure of differentiation of matrix. Irregularity of distribution of pigment. Tremendous thickening of glassy membrane. Remains of external root sheath have shrunk away from it. K-33. Ikeda. Male, 36 years of age. Approximately 1000 yds. Died on the 27th day. A.I.P. neg. HM 222.  $\times 130$ .

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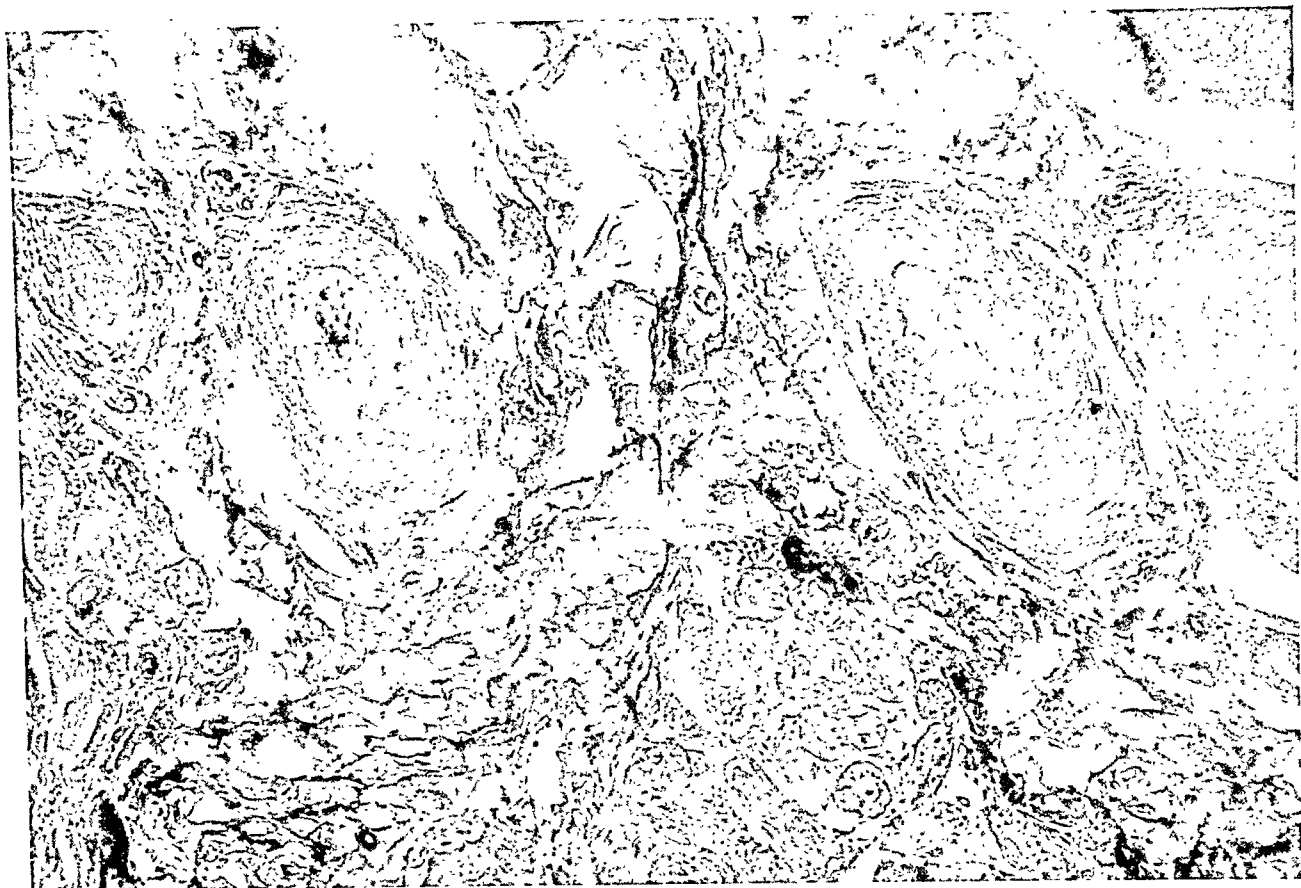
Pathology of Atomic Bomb Casualties

PLATE 163

FIG. 137. Group II. Scalp. Atrophy of hair follicles. Failure of differentiation of matrix substance. Irregular distribution of pigment. Enormous thickening of basement membrane and of glassy membrane which is proved not to be elastic tissue by Verhoeff's stain. The elastic fibers of the derma stain well. K-30. Nagashima. Male, 23 to 28 years of age (variously stated). Approximately 1000 yds. Died on the 26th day. A.I.P. neg. HM 215.

FIG. 138. Group II. Scalp. Atrophy of hair follicles. Enormous thickening of basement membranes. Atrophy of sebaceous glands. K-24. Chiba. Male, 29 years of age. Approximately 1000 yds. Died on the 25th day. A.I.P. neg. HM 294.  $\times 20$ .

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# PLATE 164

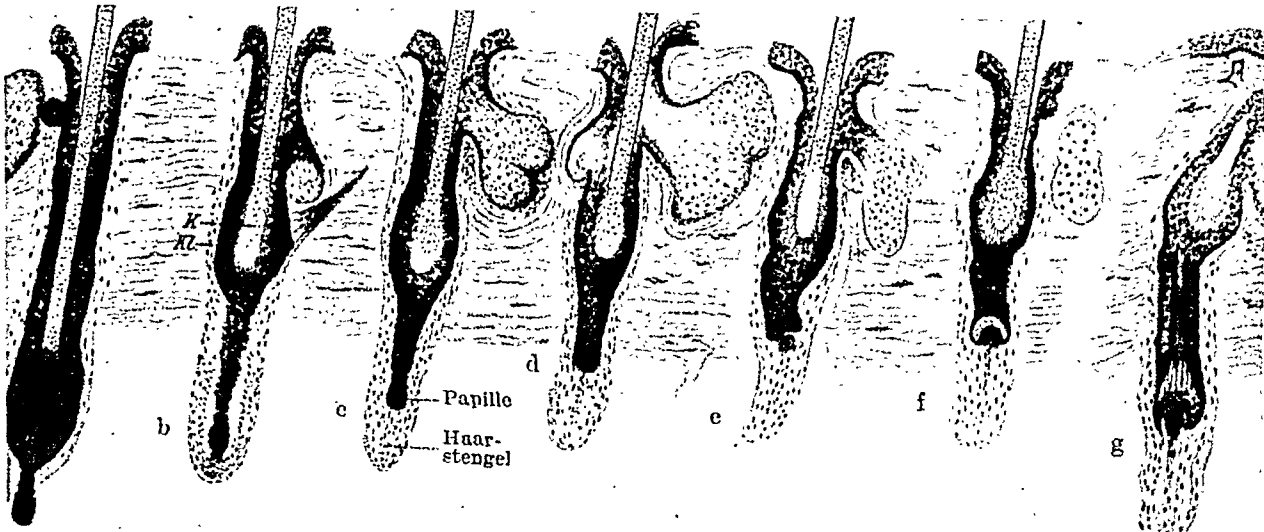
- FIG. 139. Group II. Scalp. Hair follicle. Atrophy. Hair remains fused to external root sheath. Process analogous to parakeratosis at surface. Irregularity of distribution of pigment. K-45. Akagi. Male, 28 years of age. Approximately 1000 yds. Died on the 33rd day. A.I.P. neg. HM 239.  $\times 130$ .
- FIG. 140. Group II. Hair, removed from head of partly epilated person. Tapering of shaft toward attached end; evidence of atrophy of matrix. Absence of remnants of internal root sheath. K-35. Takahashi. Male, 31 years of age. Approximately 1000 yds. Died on the 28th day. A.I.P. neg. HM 262.  $\times 50$ .
- FIG. 141. Group III. Scalp. Hair. (See also Fig. 38.) The complete follicle in longitudinal section. Atrophy of matrix. Failure of differentiation of internal root sheath. Irregular distribution of pigment. Thickening of glassy and connective tissue sheath. Remnants of atrophic shaft fill the dilated mouth of the follicle. Parakeratotic plug around this shaft. Atrophy of associated sebaceous gland. Other follicles are in process of regeneration (Figs. 134 and 142). K-14. Yamamoto. Male, 25 years of age. Approximately 1000 yds. Died on the 47th day. A.I.P. neg. HM 238.  $\times 50$ .
- FIG. 142. Group III. Regeneration of a hair is beginning at the base of the follicle by a renewed differentiation of matrix cells (in close apposition to the connective tissue papilla) and of the internal root sheath. From the same patient as Figure 141. A.I.P. neg. HM 208.  $\times 115$ .
- FIG. 143. Normal cycle of loss and replacement of a hair. At the left an external and an internal root sheath are still differentiated. At *b* the latter is no longer visible, and the former has become shrunken. There is a thickening both of the glassy and external cellular basement membrane. At *c*, *d*, and *e* there are successive stages of that process and the old hair is being extruded. At *f* the internal root sheath is being differentiated anew, and at *g* the new hair is pushing outwards in the old follicle. From Pinkus.<sup>68</sup> A.I.P. neg. HM 307.

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## THE PATHOLOGY OF HYPERPYREXIA OBSERVATIONS AT AUTOPSY IN 17 CASES OF FEVER THERAPY\*

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(*Army Institute of Pathology, Washington, D.C.*)

Heat in various forms is one of the oldest of therapeutic agents, although until recently it was employed only by local application.<sup>1</sup> Probably the first planned attempt to utilize increased body temperature as a therapeutic measure was made by Wagner von Jauregg in the malarial treatment of paresis.<sup>2</sup> Typhoid vaccine and other biologic substances have been used since, and various mechanical and electrical devices<sup>3</sup> have been constructed to serve the same purpose. Therapeutic hyperpyrexia has become an established means of treatment, not only for paresis, but for various forms of arthritis, chorea, asthma, sulfa-resistant gonorrhea, and other diseases.<sup>4</sup> As a consequence of its widespread adoption, numerous studies of the physiologic changes accompanying hyperpyrexia were undertaken. The results of some of these investigations are outlined briefly in the following paragraphs.

In the blood, hyperpyrexia produces leukocytosis; the red cell count and hemoglobin increase, the granulocytes exhibit a "shift to the left."<sup>5,6</sup> The sedimentation rate decreases,<sup>5</sup> and there is a reduction of the blood platelets.<sup>7</sup> Chemical studies show first a rise and then a fall of blood sugar.<sup>6</sup> Blood chlorides decline,<sup>6</sup> and values for nonprotein nitrogen<sup>6</sup> and lactic acid<sup>8</sup> are elevated. The plasma CO<sub>2</sub> content diminishes.<sup>9,10</sup> No changes are observed in values for calcium,<sup>11</sup> potassium,<sup>11</sup> or specific gravity.<sup>12</sup> Serum values for vitamin A decrease,<sup>13</sup> and there are conflicting reports regarding vitamin C.<sup>14-16</sup> Blood hormonal assays show increases of insulin, adrenalin, and of the antidiuretic hormone of the pituitary body.<sup>17</sup> Prothrombin<sup>7,18</sup> is diminished, and although there is no early change in plasma fibrinogen,<sup>18</sup> a late fall is observed. Miscellaneous findings include alkalinity of the urine and albuminuria<sup>6</sup>; both the heart and respiratory rates increase<sup>5,19-21</sup>; the blood pressure shows an early rise and late fall<sup>5,19-21</sup>; and counts of spermatozoa exhibit a reduction, lasting from 40 to 70 days.<sup>22</sup>

Experimentally, in rats, guinea-pigs, dogs, goats, and pigs,<sup>23-27</sup> the most common early changes are visceral congestion and disseminated focal hemorrhages in the internal organs; subendocardial hemorrhage is especially frequent. Dilatation of the right heart occurs and the blood is dark, fluid, and unclotted. There is pulmonary edema and

\* Received for publication, August 13, 1948.

hemorrhage. Later (12 to 24 hours), parenchymatous degeneration is apparent in the adrenal cortex, liver, and renal tubular epithelium. Occasionally, lower nephron nephrosis is observed.

It was recognized early that fever therapy is not without danger, and rigid controls were adopted both in the selection of patients and in the method of administration. The Council on Physical Therapy of the American Medical Association, by means of a questionnaire circulated in 1934, ascertained that 29 deaths had occurred following pyretotherapy.<sup>4</sup> Accounts of at least 20 additional fatalities have since appeared in the literature,<sup>28-33</sup> mostly as sporadic case reports with inadequately described pathologic findings. Doubtless, deaths from this cause are far more frequent than indicated in the literature, yet comparatively few attempts have been made to study the morphologic changes which occur in human beings. Although the incidence of fatality in large series of cases is admittedly low,<sup>34,35</sup> complications are not uncommon and indicate more widespread deleterious effects than are usually attributed to this form of therapy. Clinically, jaundice occurs in from 14 to 19 per cent of the patients,<sup>36</sup> circulatory collapse and transitory neurologic changes in a significant percentage,<sup>37</sup> and evanescent electrocardiographic changes are almost invariable.<sup>38</sup>

The meager evidence in the literature does not permit an adequate understanding of the pathologic effects of hyperpyrexia. Nonspecific changes reported by various authors in their efforts to avoid overlooking significant lesions further confuse the situation. Clarification is possible only by study of a relatively large group of cases.

The pertinent material at the Army Institute of Pathology consists of the clinical records and autopsy protocols together with fixed tissues and stained sections from 21 cases of fatal therapeutic hyperpyrexia. Four of these were not included in this study: the clinical information was inadequate in 2, and in 2 others there were intercurrent diseases which had produced significant modifications. Of the 17 remaining patients (all males, 3 colored, 14 white, with ages ranging from 18 to 35 years) 15 received this form of therapy because of sulfa-resistant gonorrheal urethritis. One patient had nonsuppurative arthritis, type unspecified (case 16), and one, gonorrheal urethritis treated in 1936 before the advent of sulfonamide medication (case 14). In only 2 cases was there evidence of sulfonamide intolerance or sensitivity (cases 5 and 8) and in each of these the drug had been discontinued 1 month prior to fever therapy and subsequent death. The interval between the induction of hyperpyrexia and death varied from 3 hours to 14 days. In 5 cases, fever therapy had to be discontinued because of the develop-

TREATMENT		CLINICAL										LABORATORY										PATHOLOGIC													
		CASE AND A.I.P. ACC.	HIGHEST FEVER (F)	DURATION (HOURS)	LENGTH OF SURVIVAL	SULFA DRUGS RECEIVED	TRANSFUSION	"SHOCK" DURATION	JAUNDICE	RENAL FAILURE	NEUROLOGIC CHANGES	R B C	W B C	BLOOD SUGAR (mgm%)	NPN (mgm%)	ICTERUS INDEX	CO <sub>2</sub> CONTENT (VOL%)	CREATININE (mgm%)	ALB	URINE RBC	SEROUS EFFUSION	LIVER			KIDNEY		BRAIN	HEART							
																							NON-FATTY VACUOLES	FATTY VACUOLES	CENTRAL NECROSIS	REGENERATION	PROXIMAL TUBULAR DEGENERATION	LOWER NEPHRON NEPHROSIS	EDEMA	NEURON DAMAGE	FATTY DEGENERATION	GRANULAR & HYALINE DEGENERATION	EDEMA		
1	102884	106.5	3	3 HRS	+		-			RESTLESS IRRATIONAL																									
2	99435	107.6	3	3 HRS	+		-															+								+					
3	93390	106	8	8 HRS	+		-															+	+			+		+	+	+				+	
4	82251	106.6	8 1/2	8 HRS	+		-															PLEURAL 150 cc R. 150 cc L.	+	+		+		NOT EXAMINED							
5	125672	106.8	6	11 HRS	+		-			STUPOROUS												+	+							+					
6	83533	106.7	11	11 HRS						PUPILS UNEQUAL IN REACTION												+	+			+		+	+	+					
7	93695	105	11	16 HRS	+	PASMA ONLY	-															+	+			+		+	+	+					
8	100069	106	8	16 HRS	+		+															+	+	?		+		+	+	+			+		
9	99305	105.2	7	17 HRS	+		6 HRS.			UNRESPONSIVE PUPILS DILATED			36	100		7.1						+	+			+		+	+	+				+	
10	95801	107.8	3	20 HRS	+		-															+	+			+		+	+	+				+	
11	100658	106	7	49 HRS	+		6 HRS.														++++	+	+			+		+	+	+				+	
12	100915	106.8	6-7	67 HRS	+		11 HRS.															+	+	+		+		+	+	+				+	
13	96871	105	6 1/2	73 HRS	+		?					68	75		41							PLEURAL 1000 cc R. 1000 cc L.	+	+	+		+		+	+	+		+		
14	51838	107	5	93 HRS	0		33 HRS					59 M	45500	186	33	15	38	1.3	++	+		+	+	+	?	+		+	+			+		+	
15	89594	108	6	4 DAYS	+		18 HRS.			TREMORS OF ARM AND LEG		49 M	11000	952	154	100	29-49	3.6	+++	+++		PLEURAL 300 cc R. 300 cc L.	+	+			+	+	+	+			+		+
16	79906	109	7	0 DAYS	+		?					415 M	9000		109	35			+++	+++		PLEURAL 150 cc R. 150 cc L.	+	+			+	+	+	+			+		+
17	30943	108	4	14 DAYS	+		18 HRS.					54 M	25000		308	90			+++	+		PLEURAL 150 cc R. 150 cc L. 250 cc L.	+	+			+	+	+				+		+

\* RECEIVED INTRAVENOUS GLUCOSE

TABLE I  
Clinical and Pathologic Findings in 17 Cases of Hypertension

\* RECEIVED INTRAVENOUS GLUCOSE

TABLE I  
Clinical and Pathologic Findings in 17 Cases of Hyperpyrexia

ment of such untoward symptoms as shock, coma, convulsions, or hyperexcitability. The remaining deaths occurred in cases in which symptoms developed from 2 to 36 hours following a fever treatment lasting from 3 to 11 hours. Fever had been induced by the Kettering Hypertherm Cabinet (13 cases), intravenous typhoid vaccine (3 cases), and hot baths (1 case), but the pathologic changes found at autopsy did not differ with the method of heat induction. The height of the fever and the duration of treatment are listed in Table I.

### CLINICAL FEATURES

All of the patients had been judged physically capable of enduring the treatment. The screening procedure included a complete physical examination, roentgenogram of the chest, blood count, chemical analysis of the blood, urinalysis, and electrocardiograms. Thirteen of the 17 patients were given a half-hour trial treatment which was well tolerated in each instance.

During the course of fever therapy all patients showed an increase in the pulse rate (110 to 160 per minute) and in the respiratory rate (32 to 46 per minute). Respirations were shallow, and slight to marked cyanosis was observed. The blood pressures fell somewhat following an initial transitory rise. Most patients vomited watery material one or more times. In 5 cases it was necessary to discontinue therapy because of the advent of coma, convulsions, circulatory collapse, or irrationality.

Circulatory collapse occurred in 9 cases. In 3, shock did not supervene until 2 to 36 hours had elapsed following completion of pyretotherapy. Clinical symptoms of pulmonary edema were present in 6 cases and of cerebral edema in 1 case (case 7).

Icterus occurred uniformly in all patients surviving longer than 49 hours. In the 3 who survived 4, 7, and 14 days, respectively, urinary suppression, hypertension, azotemia, and other clinical features of renal failure appeared before death.

### LABORATORY STUDIES

Laboratory studies were recorded in only a few cases. The results are presented in Table I.

### MORPHOLOGIC OBSERVATIONS

The initial effects of hyperpyrexia are clearly metabolic and after early death there is little that can be seen in the tissues. With time, however, progressive morphologic changes occur which give evidence of relatively irreversible functional injury to the cells.

*General*

Perhaps the most common feature at autopsy was the presence of moderate to marked congestion of all viscera and the brain. Congestion was present grossly in 15 cases and microscopically in all. It was most pronounced in the early cases (3 to 10 hours post-hyperpyrexia), being described by the prosectors as "marked" or "extreme," and diminished with time, so that in the cases in which survival exceeded 3 days, congestion was "slight" on gross examination or evident only microscopically.

Hemorrhages constituted another striking feature at autopsy and were present in all but 2 of the cases (Figs. 9 and 10). Their approximate incidence in various locations is shown in Table II.

TABLE II  
*The Incidence of Hemorrhage in 17 Fatal Cases of Induced Hyperpyrexia*

<i>Heart</i> .....	17	<i>Gastro-intestinal tract</i> .....	7
Subepicardial.....	10	Esophagus.....	2
Subendocardial.....	5	Stomach.....	3
Myocardial.....	2	Duodenum.....	2
<i>Brain</i> .....	4	<i>Kidney pelvis</i> .....	4
<i>Pleura</i> .....	3	<i>Miscellaneous</i> (Skin, mucous membranes of mouth, sclera, mesentery, perirenal fat).....	5

The hemorrhages, for the most part, were petechial, but in 2 cases they were ecchymotic, and in one the hemorrhagic areas in the skin measured as much as 5 cm. The frequency with which the heart was involved is corroborated by the experience of others.<sup>32,39</sup> One author<sup>32</sup> postulated that death in many of these cases was due to destruction of the conducting system of the heart by hemorrhage, a thesis which receives no support from our observations.

Transudates into one or more serous cavities occurred in 5 cases, in 4 of which survival exceeded 3 days. Pleural effusions of yellow serous fluid occurred in all 5; they were more voluminous on the right side and ranged from 150 to 1200 cc. Ascites amounting to 2200 cc. occurred in case 17; the patient had survived hyperpyrexia for 14 days. Pitting edema of the legs was present in 2 patients (cases 15 and 17), both of whom had shown clinical evidence of renal failure. Excess pericardial fluid was not observed in any case.

Jaundice was evident invariably in patients (cases 12 to 17) surviving longer than 49 hours. Its occurrence was directly related to the extent of liver damage and will be discussed when the pathologic changes of that organ are described.

Intravascular thrombosis, reported by others,<sup>39</sup> was not observed. On

the contrary, in 3 instances the prosector commented on the persistent fluidity of the blood and the almost complete absence of clotting.

Marked sickleemia occurred in 2 (cases 1 and 10) of the 3 Negro patients in this series. Neither patient had anemia or a clinical history suggestive of the sickle cell trait.

### *Liver*

The hepatic changes were the most striking of those observed. Their relation to the length of survival substantiates the assumption that the effects of hyperpyrexia are essentially physiologic and metabolic initially, and that it is only with time that morphologic alterations occur.

The livers in early cases were described as normal or congested, but in cases in which survival was as long as 8 hours post-pyretotherapy, the prosectors noticed "mottling" of the parenchyma with pale pink or gray areas. The size of the "mottled" areas increased with survival time; simultaneously the organ became less firm. The average weight of the liver when death occurred rapidly was 1850 gm. When the patient had survived 72 hours or longer, the liver was grossly enlarged (average weight, 1975 gm.), extremely soft, and uniformly yellow.

Microscopically, the earliest changes (3 hours), aside from congestion, were loss of glycogen and "cloudy swelling" of the liver cells. Cytochondrial swelling of the type described by Opie<sup>40,41</sup> was observed (Fig. 1). At 8 hours post-hyperthermia, tiny vacuoles appeared in the cytoplasm (Fig. 2), particularly in the cells of the centrolobular areas. The majority of the small vacuoles did not take the usual stains for fat and within many of them there were small eosinophilic inclusions, 2 to 5  $\mu$  in diameter. (This phenomenon of "watery vacuolization" has been produced experimentally in animals subjected to anoxia and increased hepatic venous tension.<sup>42</sup>) By 10 hours, small fatty droplets appeared in the cytoplasm (Fig. 3). These coalesced and became larger, obscuring the small non-lipid vacuoles still present. An occasional specimen showed nuclear vacuolization, but the nature of these vacuoles has not been determined. With further survival, liver injury became more manifest and necrosis was observed in the centrolobular zone. At 16 hours this was minimal but by 60 hours some 60 per cent of the central part of the lobule was necrotic (Fig. 4) and hyperemic. If survival was sufficiently prolonged, reparative changes became increasingly prominent. At 7 days, phagocytosis of cellular detritus by macrophages was conspicuous (Fig. 6); polymorphonuclear leukocytes played only a minor rôle in this process. The relatively undamaged cells at the periphery of the lobule underwent active regeneration as evidenced by mitotic figures,

multinucleated liver cells, nuclear enlargement, and prominent nucleoli (Figs. 5 and 8). Proliferation of biliary channels occurred simultaneously. Morphologically, such a liver may resemble those found in fatal cases of epidemic hepatitis,<sup>43</sup> but the sparsity of inflammatory cellular reaction and the lack of liver atrophy are helpful differential features. Since the liver damage in fatal cases is presumably greater than that sustained by survivors of pyretotherapy, changes of this magnitude would not be expected among the latter. No appreciable fibrosis was present even in the case of longest duration (14 days) (Figs. 7 and 8).

Jaundice occurred only in patients surviving longer than 49 hours; in each of these the centrilobular destruction involved 40 per cent or more of the liver lobule.

#### *Central Nervous System*

The brain was examined in 16 cases; weights ranged from 1260 to 1700 gm. and averaged 1480 gm. Conspicuous congestion of the blood vessels of the meninges or underlying brain was recorded in 13 instances, edema was considered a feature in 9, and a cerebellar pressure cone in 1. Petechiae or small ecchymotic areas were present in 4. They involved the corpus callosum, the caudate nucleus, the periventricular region (Fig. 10), and the white matter of the cerebral cortex in close proximity to the gray matter. Focal subarachnoid hemorrhages occurred over the cerebellar hemispheres.

Microscopically, perivascular edema (Fig. 11) was present in the majority of the brains and "ring" hemorrhages in 4 (cases 3, 12, 15, and 16).

The parenchymal damage incurred by the central nervous system was most striking and constant in the cerebellum. When death occurred in less than 24 hours, focal neuronal degeneration was present in the Purkinje cell layer (Figs. 9 and 14), but the molecular and granular layers were not appreciably altered. With survival beyond 24 hours, degeneration was progressively more severe and was featured by edema and moderate reactive glial proliferation. In the patients who died later than 7 days after pyretotherapy, most of the Purkinje cells had disappeared and the few remaining were deeply stained and pyknotic. The dentate nucleus showed neuronal changes similar to those in the cortex which are described in the following paragraph.

Changes in the nerve cells of the cortex were present even in the earliest cases, of 3 hours' duration. In all cases the alterations were widely scattered and affected small focal areas. While the majority of the neurones were well preserved in these, some were swollen, exhibiting chromatolysis and karyolysis, and others were shrunken, with deeply



staining eosinophilic cytoplasm and pyknotic nuclei (Fig. 13). At this stage there was no apparent reaction on the part of the glia. Subsequently, disappearance or disintegration of nerve cells was observed in the focally damaged areas (Figs. 14 and 15). Glial reaction was not observed in the cerebrum or brain stem; presumably degenerative changes were too sparse and too widely scattered. In case 10, in which small vessels were occluded with masses of sickled red cells, definite areas of ischemic necrosis were most prominent in the deeper layers of the cerebral cortex and in the periventricular part of the thalamus (Fig. 12).

Similar but less severe neuronal changes were present throughout the basal ganglia and brain stem. No sections of spinal cord were available for study, but cellular damage in a fortuitously sectioned perivesical autonomic ganglion in one case suggested that the changes in nerve tissue might be widespread.

In 2 cases (10 and 12) the leptomeninges exhibited a very slight pleocytosis, the result of activation of histiocytes of the arachnoid trabeculae. According to Haymaker,<sup>44</sup> "similar changes have been observed in a great variety of disorders including anoxia of various types and toxic-infectious states."

### *Kidney*

Renal changes were less constant than those already noted in the brain and liver. The kidneys were regarded as normal in those patients who died within 24 hours. Beyond this period, they were increased in weight, averaging 282 gm. each, and were described as pale, swollen, and somewhat softened. Petechiae were present beneath the pelvic mucosa in 4 (8 to 98 hours' duration) and in the perirenal fat in one.

Microscopically, congestion was marked if death occurred in less than 24 hours and slight interstitial hemorrhage was observed in one of these cases. With longer survival this feature was obscured by the more pronounced parenchymal changes. Parenchymatous damage was scanty in cases terminating rapidly. In the 7 cases in which death occurred within 12 hours, cloudy swelling of the tubular epithelium was the only consistent change and in places the swollen granular tubular cells occluded the lumen. In 2 of the 4 cases of 12 to 24 hours' duration, widespread karyolysis, karyorrhexis, and fatty degeneration involved the proximal convoluted tubules. Granular debris in the tubules appeared to have been derived from disintegration of the lining cells. Although absent in the early cases, in 5 of the 7 cases in which death was delayed for more than 48 hours the changes of lower nephron nephrosis<sup>45</sup> were apparent (Fig. 17). Pigmented casts were present within the lumina of distal convoluted tubules which showed progressive disintegration and detach-

ment of the lining epithelium. Concurrently, interstitial edema and cellular infiltration (largely lymphocytic) appeared. Numerous hyaline casts indicated a high degree of albuminuria and tubular stasis. Interstitial infiltrates were observed also in 2 of the earlier cases in which more widespread lesions indicated sulfonamides as the causative factor.<sup>46,47</sup> Three patients who survived 4, 7, and 14 days, respectively, died of renal failure with hypertension, azotemia, and oliguria.

### *Heart*

The majority of the hearts in this series fell within the normal limits of weight; 2 showed an unexplained, probably pre-existent, increase to 460 and 510 gm. In 3 cases there was dilatation of the right auricle and ventricle. Hemorrhages were common (Table II) and occurred with equal frequency in the cases of short and long duration. The hemorrhages were petechial and occurred most commonly in the subepicardial tissues at the base of the heart. In 4 cases there were hemorrhages in the subendocardial layer of the interventricular septum, but microscopic examination revealed no involvement of the conduction bundles.

The microscopic changes in the heart were inconstant and, while more common in the cases of longer duration, were present also when survival was less than 24 hours. Focal degenerative myocardial changes were observed in 9 hearts: they were granular and hyaline in 5 (Fig. 18), fatty in 2, and lesions of both types were present in 2 others. Three hearts were the seat of slight stromal hyperplasia and cellular infiltration unrelated to the muscle lesions. These were considered results of sulfonamide administration, since infiltrates were present also in other organs.<sup>46,47</sup> Interstitial edema unrelated to renal failure occurred in 4 cases, fragmentation and rupture of muscle fibers in 2.

### *Lungs*

The lungs usually were heavy, weighing together as much as 2560 gm. with an average weight of 1575 gm. In only one instance were the lungs of normal weight; in the others they were filled with edema fluid and blood. There were petechiae beneath the pleura in 5 cases. Microscopic examination merely substantiated the gross findings of edema, hemorrhage, and congestion. The changes of terminal bronchopneumonia were present in 2 cases.

### *Spleen*

The spleen usually was enlarged, the average weight being 270 gm.; the range was from 160 to 530 gm. The spleen was soft and flabby with

an intensely congested, frequently diffuent pulp. In one case with sickle-  
mia the perifollicular hemorrhages reported by Rich<sup>48</sup> were observed.

### *Adrenals*

Grossly, the adrenals were unaltered except for post-mortem autolysis of the medulla in a few cases. Microscopically, engorgement of the cortical sinusoids was obvious in cases of early death. The earliest parenchymatous change was in the lipid of the adrenal cortex. Normally, in the fasciculate zone, intracellular lipid is present in the form of tiny uniform droplets, but as early as 3 hours after fever induction it was noted that these droplets had coalesced, appearing as large irregular vacuoles in the histologic sections (Fig. 20). The vacuoles increased in size progressively until the cells disintegrated (Fig. 21). The loss of these cells and their replacement by fluid resulted in the formation of "tubular" structures similar to those reported by Rich<sup>49</sup> in fulminant infections (Figs. 22 and 23). In cases in which survival exceeded 24 hours, the cortical cells had a homogeneous eosinophilic cytoplasm without lipid. Small foci of acute necrosis in the cortex were evident in 3 of the cases of 1 to 3 days' duration. This "tubular" change was no longer exhibited in any of the 5 which had a survival period of more than 3 days after fever therapy, suggesting that it is a form of reaction to an acute injury.<sup>50</sup> The adrenal medulla appeared normal in every case, but the other changes, although nonspecific, contradicted the statements by others<sup>39</sup> that no significant injury to the adrenals is caused by hyperthermia.

### *Testis*

Gross abnormalities of the testes were not observed, although the prosector in one case described "diminished consistency." Microscopic changes, not present if death occurred within 8 hours, were noted frequently. Spermatogenesis was greatly decreased and assumed an abnormal pattern. Giant multinucleated cells were formed in the walls of the tubules (Fig. 24) and subsequently found their way into the lumina (Fig. 25). Such cells are not a specific effect of heat injury since they have been reported in deficiencies of vitamin A or E and in inanition.<sup>51-53</sup> It seems reasonable to regard them as abnormal forms resulting from the failure of cytoplasmic cleavage to keep pace with nuclear division. With progressive impairment of spermatogenesis (Fig. 26), the testicular tubules may consist solely of Sertoli cells. In the majority of cases the intertubular stroma was edematous but the interstitial cells were unaffected and there was no evidence of inflammatory infiltration. Whether recovery of spermatogenesis occurred could not be ascertained in these

cases. A clinical study, however, indicates that the spermatozoa counts return to normal in from 40 to 70 days following hyperpyrexia.<sup>22</sup>

### *Other Organs*

*Gastro-intestinal Tract.* Hematemesis had been observed in 3 patients prior to death, but the hemorrhages encountered in 7 cases at autopsy were punctate and confined to the mucosa (Table II). Edema of the submucosa was noted occasionally and congestion was prominent in virtually all cases. Ulceration of the esophagus was present in 2 cases; one of these patients had been subjected to repeated passage of a stomach tube (case 14).

*Bone Marrow.* Histologic study of bone marrow was limited to 5 cases and none of these showed essential alteration of hematopoiesis. In one case of sicklemlia (case 10), foci of fat necrosis occurred in the bone marrow (Fig. 19). In view of the limited nature of the material, the absence of changes must not be regarded as a denial of injury to megakaryocytes as reported in heat stroke.<sup>54</sup>

Sections of *pancreas, thyroid gland, skeletal muscle, urinary bladder, and prostate* contained no significant lesions that might be attributed to hyperpyrexia.

A summary of the more important clinical, laboratory, and pathologic findings is presented in Table I.

### DISCUSSION

In fatal febrile conditions, it is impossible to segregate the morphologic effects of fever from those of the underlying process. As a consequence, the pathologic changes have never been clearly defined, although clinicians have long been aware of the danger of high body temperature, one of the commonest symptoms of disease. It seems particularly important, therefore, to report the observations in these 17 fatal cases which, although death was accidental, illustrate essentially the morphologic changes of controlled hyperpyrexia unmodified by other disease processes.

The explanation of the widespread effect of hyperpyrexia involves consideration of several factors. Kopp and Solomon<sup>55</sup> regarded shock as the sole pathogenic factor, and it is true that hemorrhages, serous transudates, focal myocardial degeneration, centrolobular hepatic necrosis, tubular degeneration and necrosis in the adrenal cortex, and lower nephron nephrosis have all been observed. But circulatory collapse occurred in only 9 of the 17 cases which we have studied. Moreover, their thesis merely substitutes one question for another, since the cause of

shock itself remains unexplained. We subscribe to Hartman's<sup>56</sup> view that anoxia constitutes the prime (but not the sole) injurious factor in hyperpyrexia, although we recognize that it may also occur in shock. Circulatory collapse, when present, serves only as an augmenting factor and adds anoxia of the stagnant variety to the anoxia already present. Hartman noted the similarity of the pathologic lesions following fever therapy and those due to prolonged asphyxia, as in carbon monoxide or nitrous oxide poisoning. He demonstrated experimentally that severe anoxia was produced constantly in animals by induced fever. Although oxygen determinations of the blood were not made in this series, the existence of significant anoxia was indicated by the constant occurrence of cyanosis, clinically, and by the presence of sickled red cells in the tissues at two of the post-mortem examinations. Since the routine laboratory studies had failed to reveal evidence of the sickle cell trait during life, we must infer a severe and prolonged anoxemia to account for the unmasking of the inherent cellular defect.

It is estimated that a body temperature of 106° F. increases the metabolic rate by 50 per cent,<sup>57-59</sup> and oxygen utilization proportionately. Yet the physiologic mechanisms for furnishing oxygen operate at reduced efficiency. At increased temperatures the oxygen-combining capacity of hemoglobin is diminished. Alkalosis induced by the hyperpnea of fever<sup>58-64</sup> results in an increased stability of oxyhemoglobin and impairs the release of oxygen to the tissues. Finally, the increased rate of blood flow reduces the time available for oxygen transfer.<sup>19,65</sup> Actual measurements by Cullen, Weir, and Cook<sup>66</sup> have shown that arterial oxygen saturation is decreased by approximately 25 per cent (comparable to that attained by ascent to an elevation of 17,500 feet) while venous oxygen saturation is increased to a fairly high level, thus demonstrating both a decreased supply and decreased utilization or delivery of oxygen. These conditions are not altered by oxygen administration and it is apparent that such therapy can only ameliorate,<sup>60</sup> and not prevent, deleterious effects. It is known that anoxia increases capillary permeability greatly (fluid passes through capillary walls at four times the normal rate after 3 minutes of anoxia<sup>67,68</sup>), and it seems likely that this accounts for the non-lipoid vacuoles in the cells of the liver and other organs. Similar changes have been produced experimentally in rabbits and occur with great rapidity.<sup>69</sup> They have been observed also in human material following accidental death from anoxic anoxia under conditions precluding survival for more than fractions of an hour.<sup>42,69-75</sup> The hydrostatic pressure of the blood, of major importance initially, becomes less essential as increase of survival time permits degradation of cytoplasmic

constituents. The latter process must certainly be associated with increased osmotic activity. It is to be expected that the changes would be most striking in the central portion of the hepatic lobule, considering that cells in that position are most remote from the arterial blood supply. Although the mechanism by which anoxia causes neuronal degeneration is not known, the sensitivity of the brain to oxygen lack is well established. It is logical to believe that identical lesions occurring in hyperpyrexia and in anoxic anoxia<sup>74,75</sup> are produced by the same mechanism. The site of the anatomic changes in the brain, though, may vary with the type of anoxia; according to Haymaker<sup>44</sup> the globus pallidus sustains the most severe damage in carbon monoxide poisoning, whereas the striatum bears the brunt of the injury in cyanide poisoning. The implication is that the metabolic injury producing cellular damage is more complex than simple oxygen lack.

Less measurable but none the less real alterations presumably occur among the cellular enzymes to explain the accumulation of fat in liver cells, myocardial fibers, and renal epithelium. The unraveling of the mechanism of carbohydrate utilization within the past decade has permitted an insight into the importance of complex interlocking and interrelated enzyme systems in cellular (and body) metabolism. Since enzymatic reactions are characteristically sensitive to alterations in temperature and pH, prolonged fever may be expected to have some influence on these vital activities. If sufficient damage is sustained, cellular metabolism will no longer be possible and necrosis will occur. With slighter degrees of injury the defect may become manifest as inability of the cell to utilize a material or materials which it ordinarily metabolizes. Using the conventional histologic technics, fat is the most readily demonstrable of these substances although there is no assurance that it is the only one. It seems likely that the inability of spermatogonia to divide properly represents another type of cellular metabolic inadequacy. The impaired capacity of the liver to form prothrombin and fibrinogen<sup>18</sup> is clearly attributable to a "bottleneck" in the cellular "production line." In this way the hemorrhagic tendency and decreased coagulability of the blood are aggravated. Other factors contributing to the latter are the direct destructive effects of heat on prothrombin,<sup>76</sup> platelets, and megakaryocytes,<sup>7,54</sup> increased capillary permeability,<sup>67</sup> and diminished capillary resistance.<sup>77</sup>

It is somewhat more difficult to explain the occurrence of lower nephron nephrosis in some of these patients. Transfusions or sulfonamides, either of which may cause kidney lesions of this type,<sup>45</sup> were given singly or together. However, since the transfusions were all compatible

and reactionless and the other viscera showed no morphologic evidence of sulfonamide sensitivity, it is probable that the renal injury was directly related to hyperthermia. Lower nephron nephrosis in 19 cases of heat stroke was unassociated with significant hypotension in 6.<sup>54</sup> Anoxemia must certainly be implicated in its causation as well as the cellular metabolic derangements more directly related to fever. Degradation products from quantitatively significant tissue necrosis, especially of the liver, are present to impose an additional burden upon renal function. Shock, should it supervene under such circumstances, would further diminish the likelihood of the kidneys escaping unscathed.

Finally, the similarity of the changes described to those occurring in thyroid crisis,<sup>78-82</sup> the postoperative liver-death syndrome,<sup>83</sup> and heat stroke,<sup>54</sup> all conditions in which hyperpyrexia is a prominent feature, would suggest a common mechanism of injury.

#### SUMMARY

Seventeen fatal cases of therapeutic hyperpyrexia have been reviewed to ascertain the nature of any pathologic changes that might have occurred. The underlying disease in each instance was of a type not usually associated with more than local tissue changes. Therefore, except for the complicating factors considered in the text, morphologic alterations were regarded as the result of the controlled fever.

Congestion and purpuric hemorrhages were the rule. Patients who did not die within 48 hours after the fever also exhibited jaundice. Microscopically, necrosis of cells and other degenerative changes were observed in the brain, heart, liver, kidney, adrenal glands, and testes. The alterations, especially in the liver, were progressively more severe as the survival time increased. It is inferred that the absence of visible change when death is prompt merely expresses the limitations of current histologic technics.

Anoxia and deleterious effects of elevated temperature upon essential cellular enzymes and enzyme reactions are probably the essential factors in producing these pathologic effects. Fever in many conditions besides pyretotherapy may produce similar lesions.

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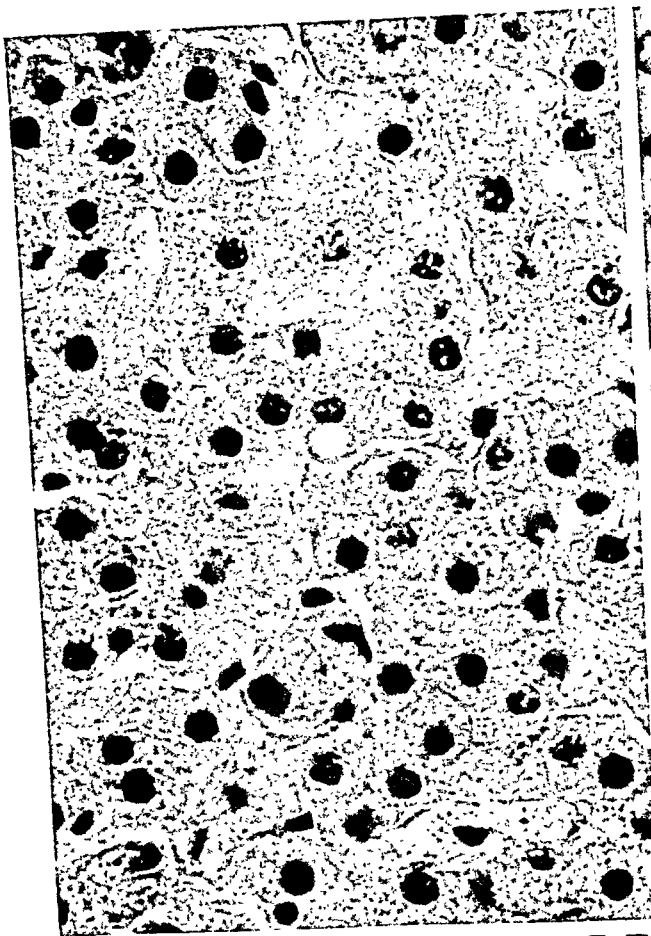
#### DESCRIPTION OF PLATES

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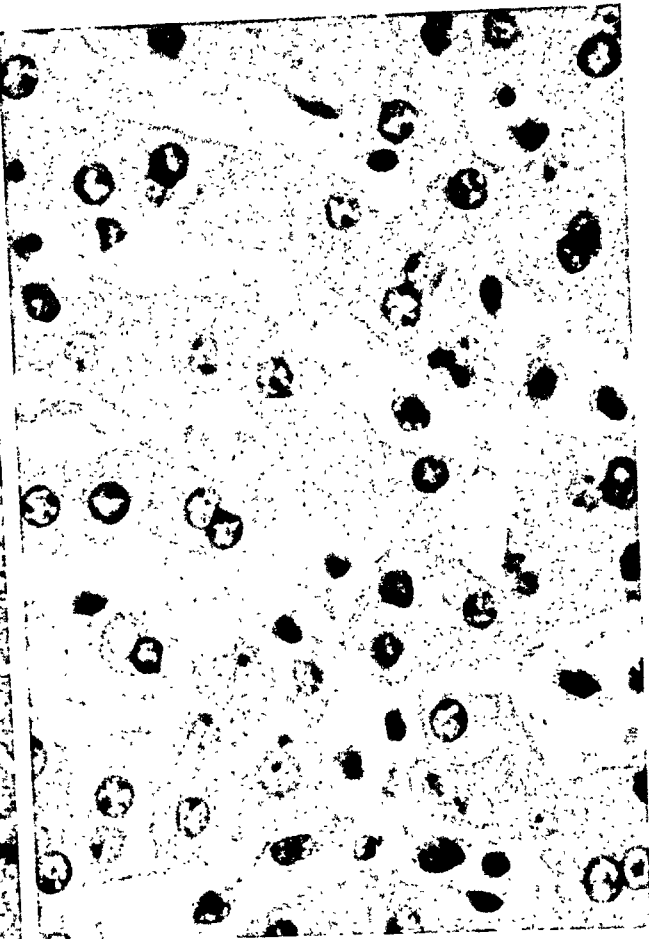
##### PLATE 165

- FIG. 1. Case 1, Army Institute of Pathology accession no. 102884. Liver, demonstrating cellular granularity (cytochondrial swelling) after 3 hours of hyperpyrexia.  $\times 435$ . Negative no. 103922.
- FIG. 2. Case 3, A.I.P. acc. 93390. Non-lipid vacuolization of liver cells 8 hours after onset of hyperpyrexia.  $\times 515$ . Neg. 103909.
- FIG. 3. Case 8, A.I.P. acc. 10169. Large fatty and small non-lipid vacuoles of the liver cells.  $\times 600$ . Neg. 103722.
- FIG. 4. Case 15, A.I.P. acc. 89594. Centrolobular degeneration of liver 100 hours after onset of fatal hyperpyrexia.  $\times 100$ . Neg. 74693.

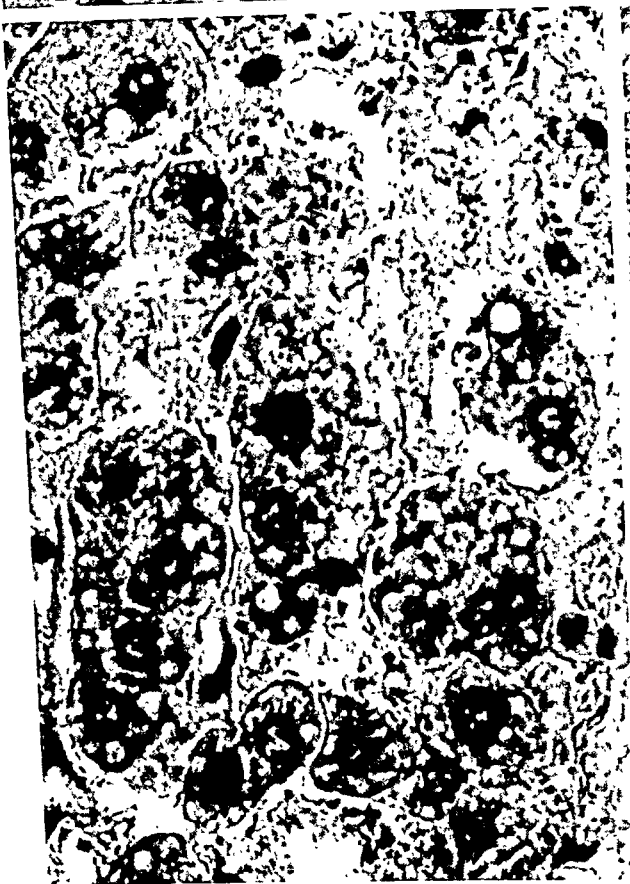
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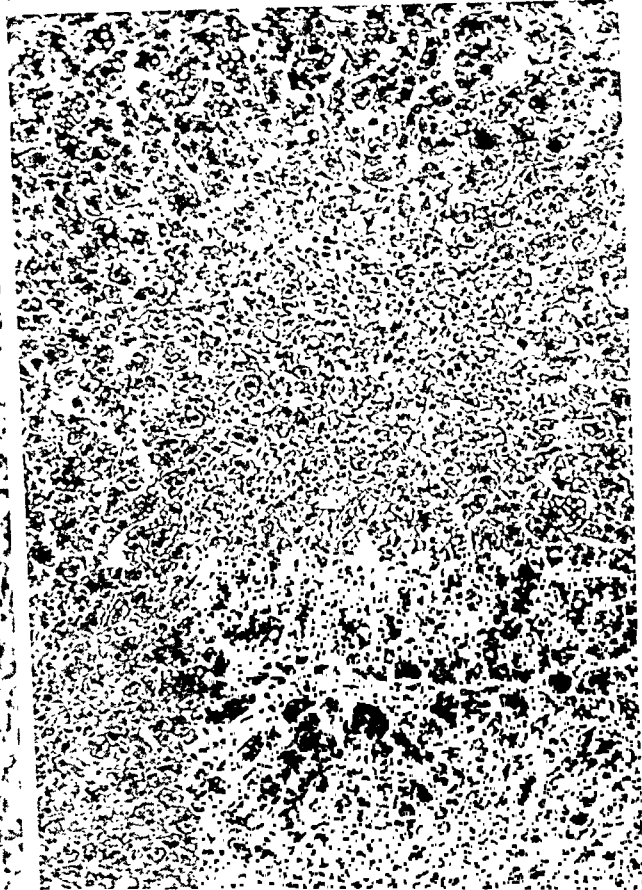


PLATE 166

- FIG. 5. Case 16, A.I.P. acc. 79906. Mitotic figures in liver showing regenerative activity 7 days after therapeutic fever.  $\times 475$ . Neg. 103706.
- FIG. 6. Case 16, A.I.P. acc. 79906. Focal accumulation of macrophages replacing liver cells.  $\times 395$ . Neg. 103705.
- FIG. 7. Case 17, A.I.P. acc. 130943. Considerable loss of liver cells, stromal collapse, and hyperplasia of the residual cells have occurred.  $\times 70$ . Neg. 103925.
- FIG. 8. Case 17, A.I.P. acc. 130943. From the same section as Figure 7, but at a higher magnification. Of note are the mononuclear character of the leukocytic response, the proliferating bile ducts, and the bile "thrombi."  $\times 275$ . Neg. 103926.

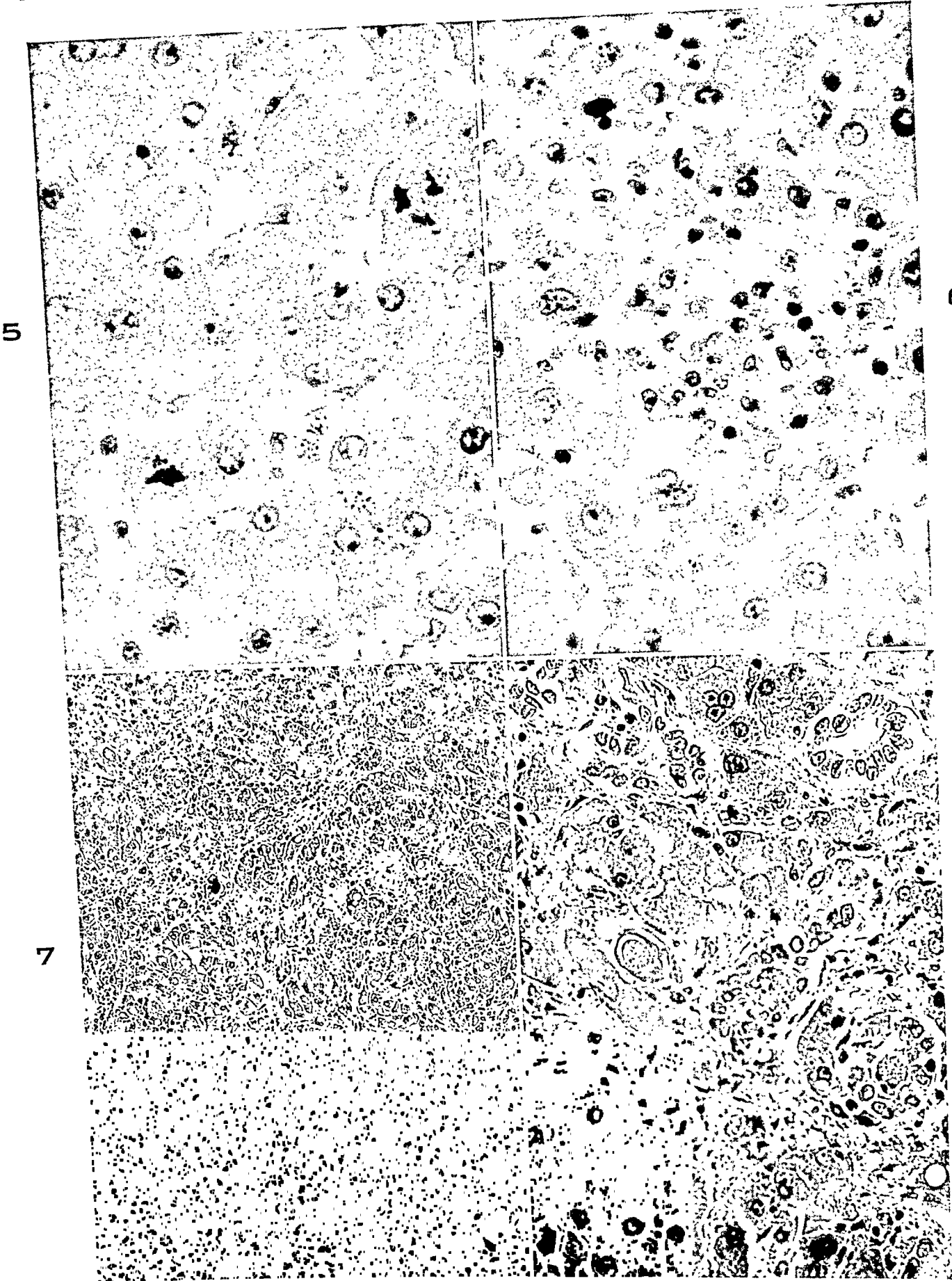


PLATE 167

- FIG. 9. Case 15, A.I.P. acc. 89594. Cerebellum 100 hours after fever therapy. Subarachnoid hemorrhage and degenerative changes are seen in the Purkinje cell layer.  $\times 75$ . Neg. 103724.
- FIG. 10. Case 15, A.I.P. acc. 89594. Hemorrhages beneath the subependymal cell plate of the third ventricle 100 hours after fever therapy.  $\times 15$ . Neg. 74071.
- FIG. 11. Case 17, A.I.P. acc. 130943. Cerebrum 14 days after fever therapy. There is perivascular edema of the subcortical white matter.  $\times 75$ . Neg. 103711.
- FIG. 12. Case 10, A.I.P. acc. 95801. Focal degeneration in the caudate nucleus 20 hours after artificial fever. Sicklemia had not been recognized during life.  $\times 65$ . Neg. 103698.

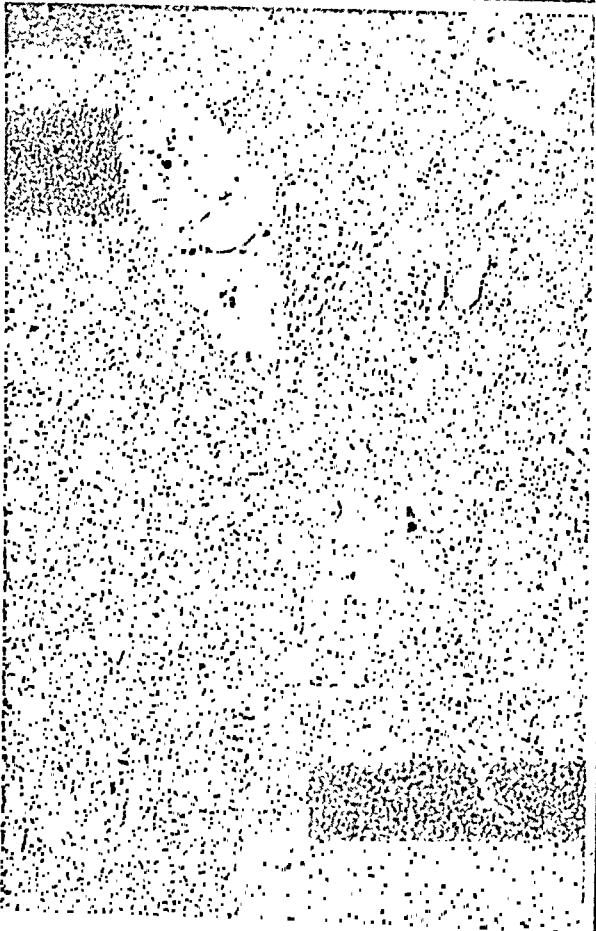
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Gore and Isaacson

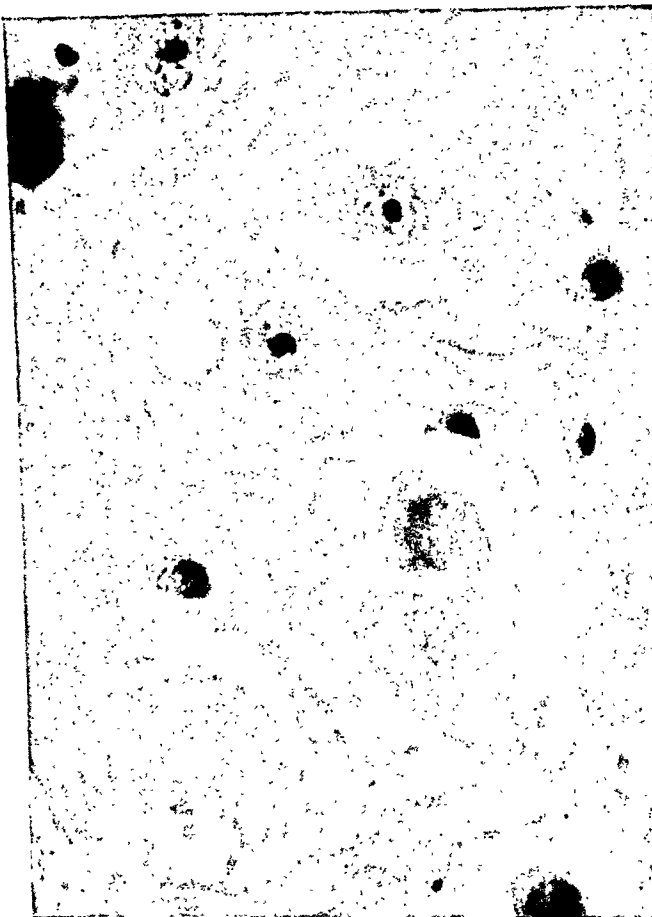
Pathology of Hyperpyrexia



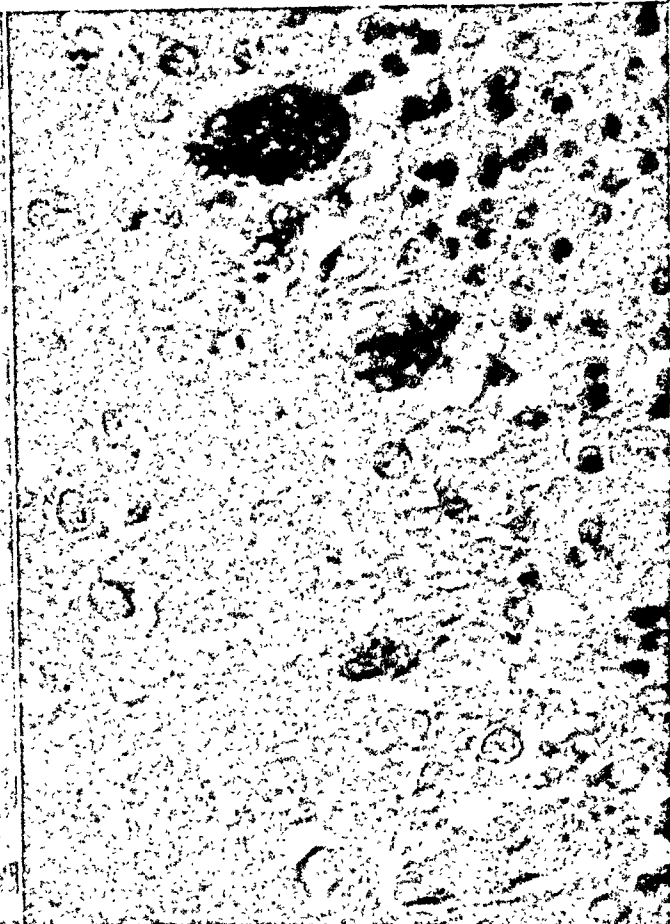
PLATE 168

- FIG. 13. Case 1, A.I.P. acc. 102884. Inferior olivary nucleus, medulla, 3 hours after the onset of artificial fever. A few of the nerve cells exhibit karyolysis, others are pyknotic.  $\times 475$ . Neg. 103729.
- FIG. 14. Case 7, A.I.P. acc. 93695. Purkinje cell layer of the cerebellum 12 hours after the onset of hyperpyrexia. There is loss of nuclear structure in all of the Purkinje cells in this field.  $\times 515$ . Neg. 103716.
- FIG. 15. Case 10, A.I.P. acc. 95801. Hippocampus 100 hours after fever, showing acute degenerative changes in the cells of Sommer's sector.  $\times 275$ . Neg. 104346.
- FIG. 16. Case 15, A.I.P. acc. 89594. Hypoglossal nucleus showing both cellular lysis and pyknosis 100 hours after artificial fever.  $\times 355$ . Neg. 103735.

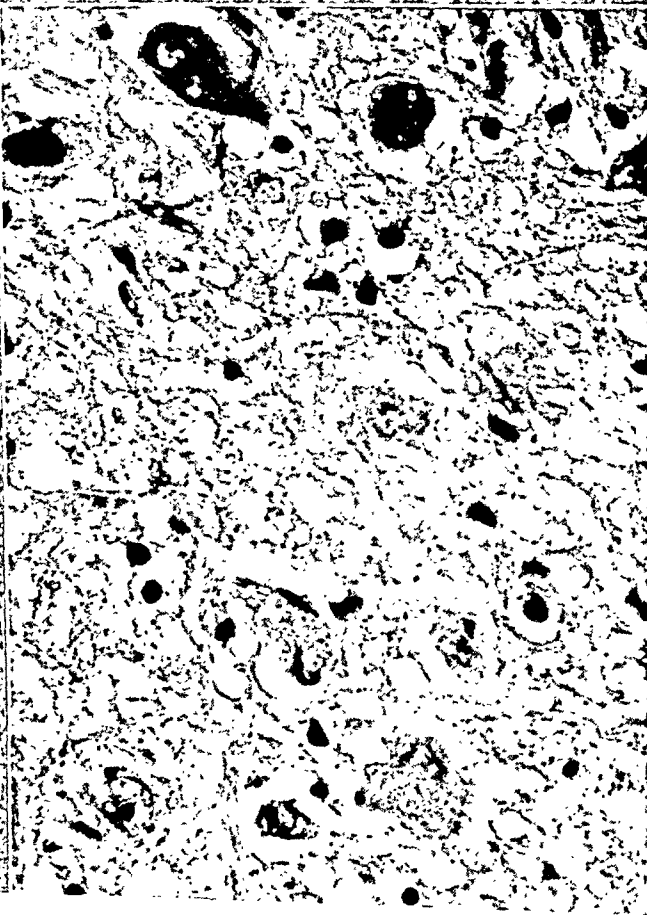
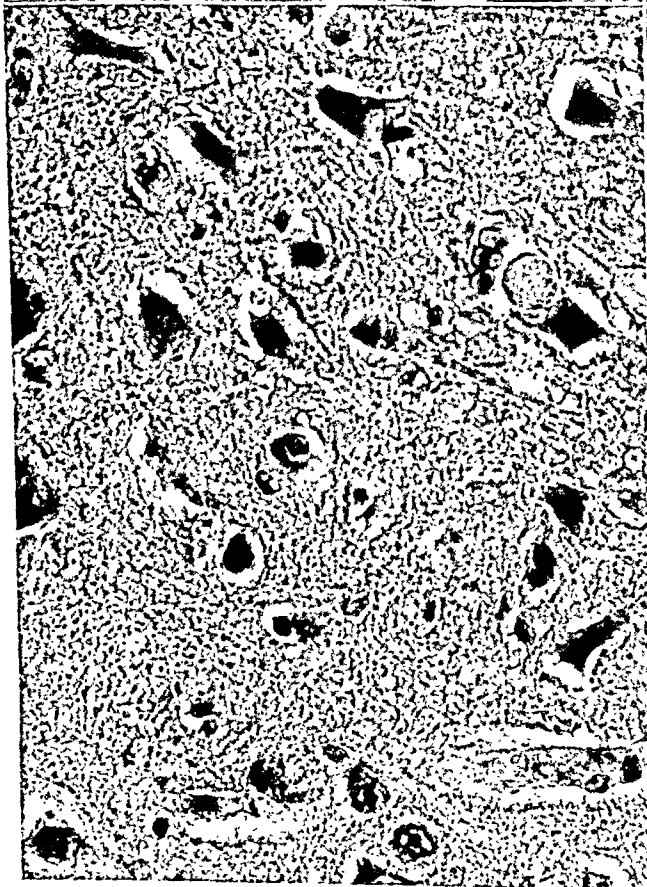
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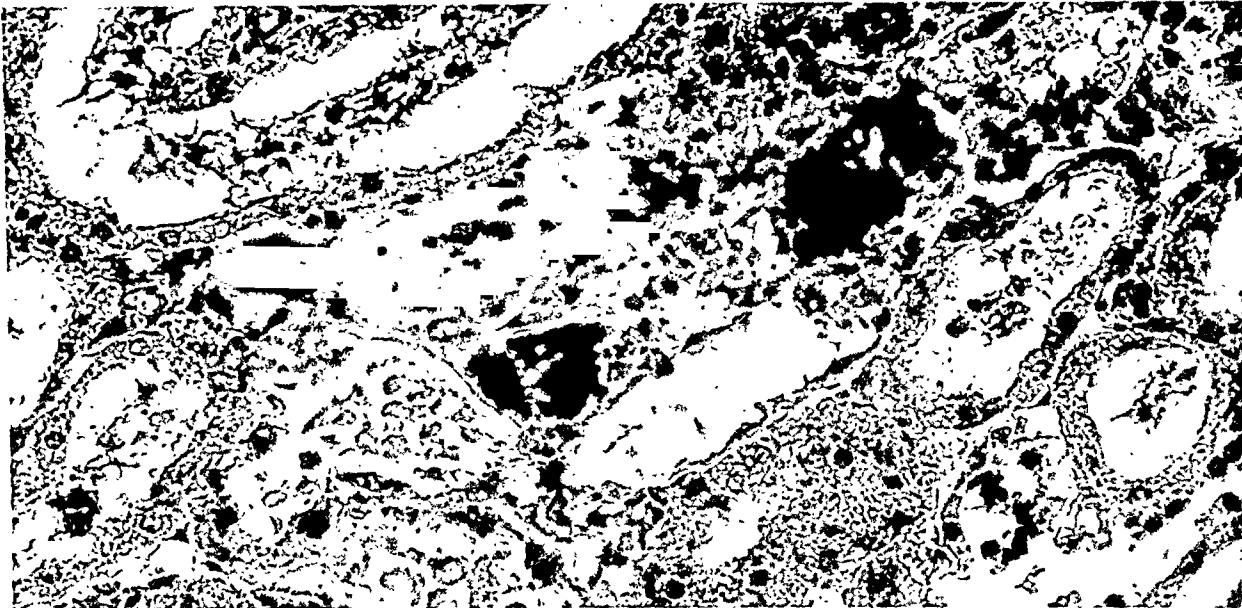
Gore and Isaacson

Pathology of Hyperpyrexia

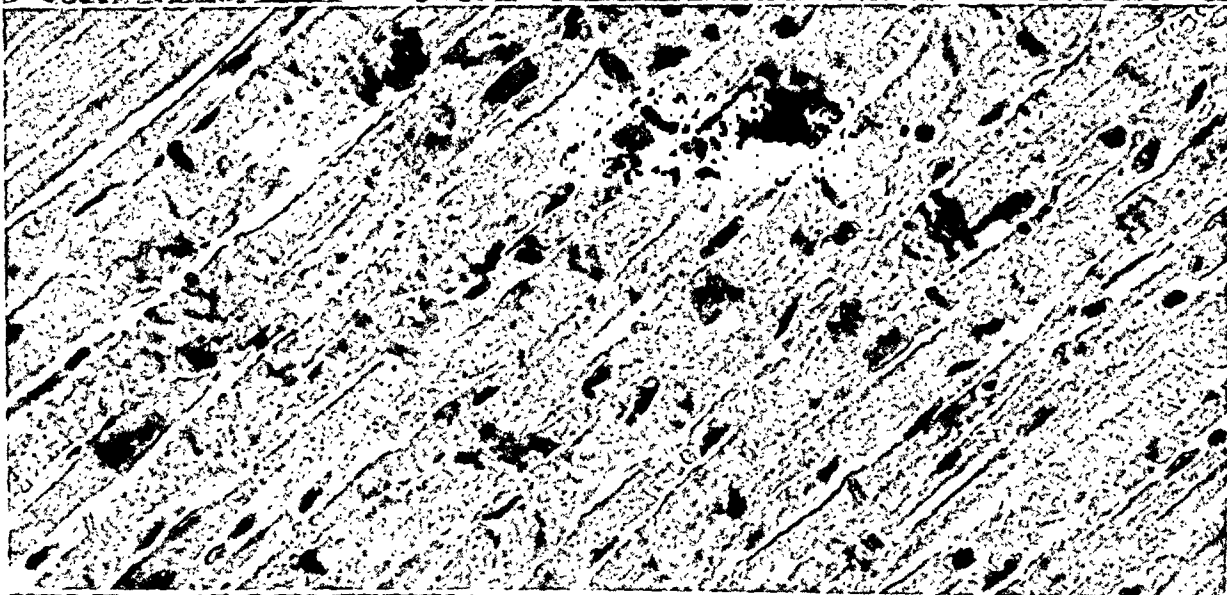
PLATE 169

- FIG. 17. Case 15, A.I.P. acc. 89594. Distal nephron nephrosis 100 hours after onset of hyperpyrexia.  $\times 350$ . Neg. 74686.
- FIG. 18. Case 10, A.I.P. acc. 95801. Focal hyaline and granular degeneration of myocardium 20 hours after onset of hyperpyrexia.  $\times 400$ . Neg. 103701.
- FIG. 19. Case 10, A.I.P. acc. 95801. Focal area of fat necrosis in the bone marrow 20 hours after onset of hyperpyrexia. The sickle cell trait had not been recognized during life.  $\times 150$ . Neg. 103695.

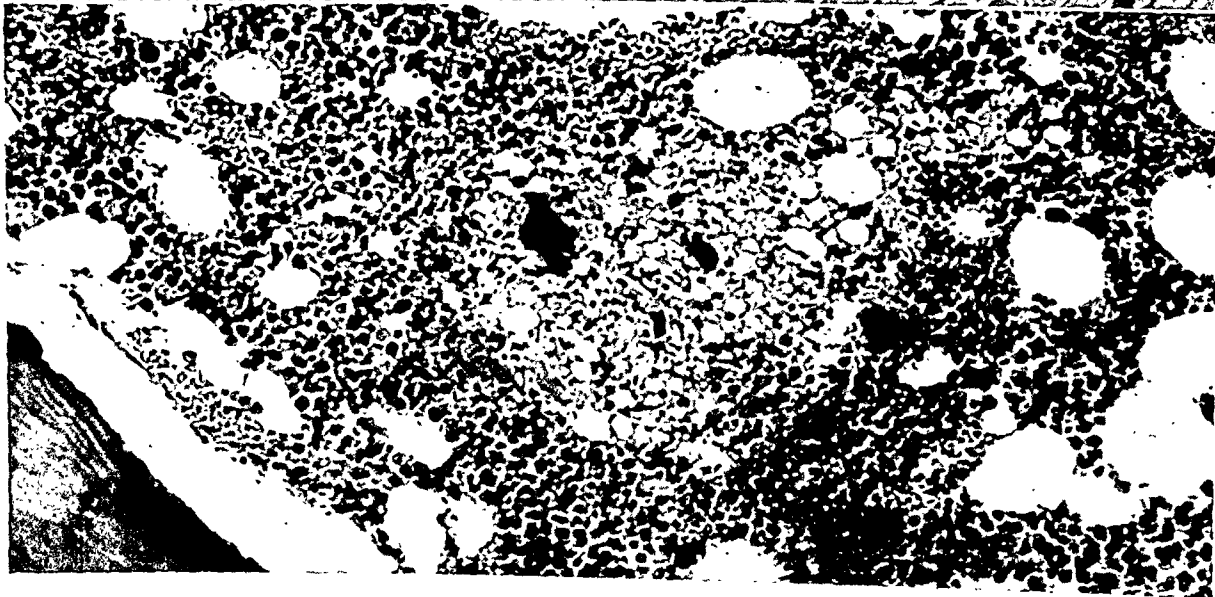
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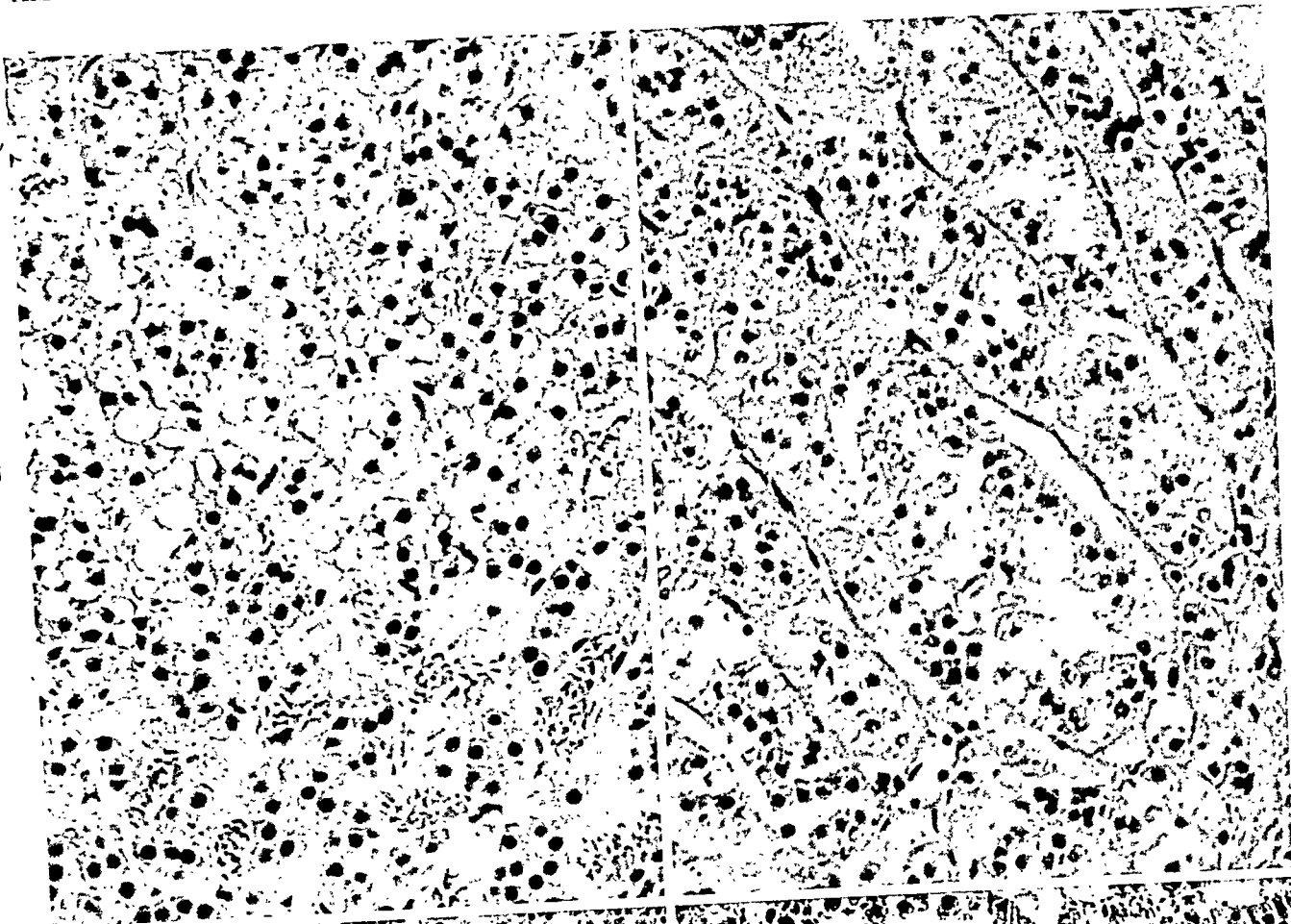
Gore and Isaacson

Pathology of Hyperpyrexia

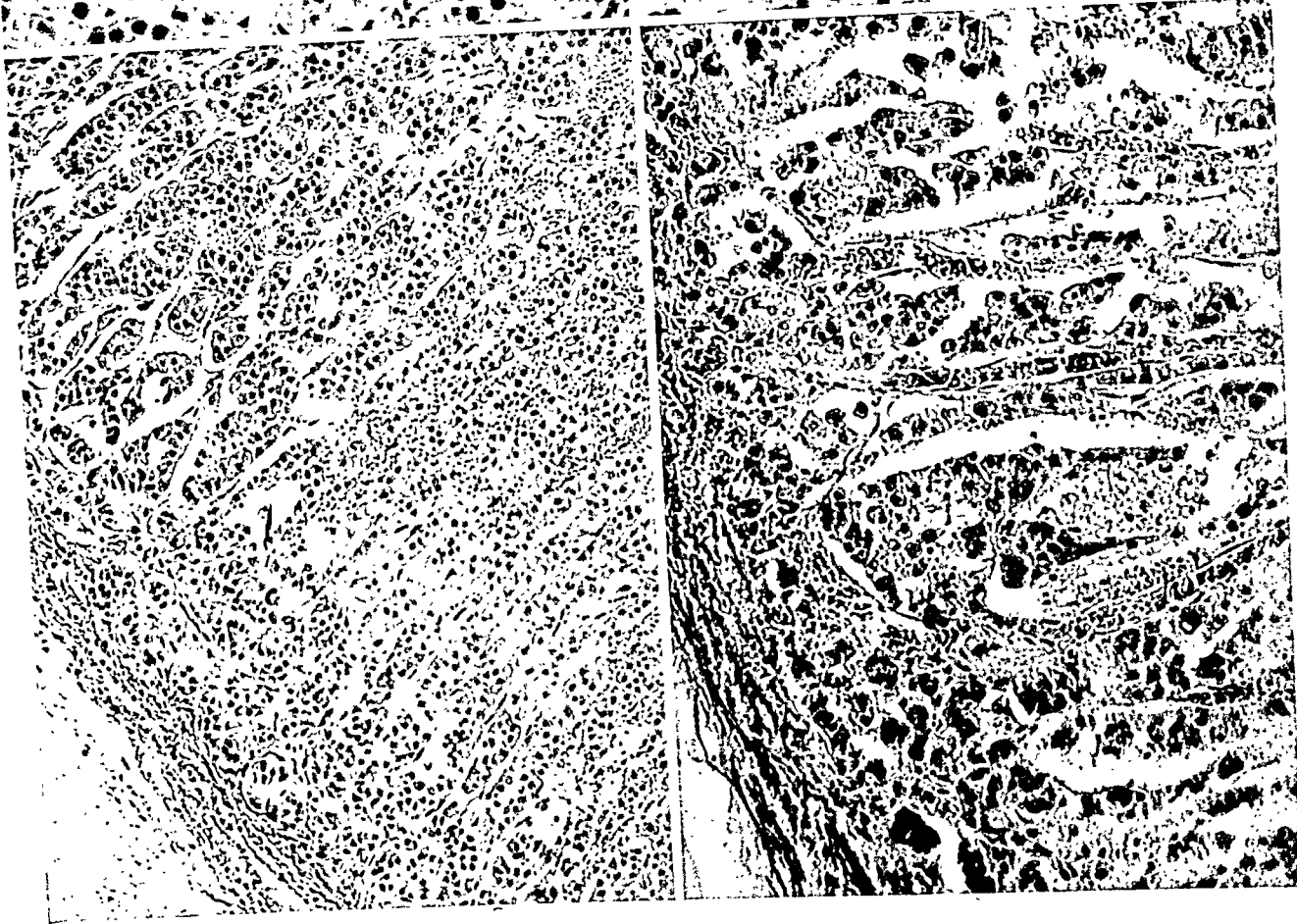
PLATE 170

- FIG. 20. Case 11, A.I.P. acc. 102884. Adrenal cortex 3 hours after the onset of fatal hyperpyrexia. The normally small lipoid droplets have coalesced to form large, irregularly sized vacuoles. Of note are the sickled red cells in the vessels.  $\times 214$ . Neg. 103728.
- FIG. 21. Case 2, A.I.P. acc. 99435. Early stage of "tubular" degeneration,  $3\frac{1}{2}$  hours after onset of fatal hyperpyrexia. Necrotic cells are visible in the spaces. Adrenal cortex.  $\times 214$ . Neg. 103726.
- FIG. 22. Case 3, A.I.P. acc. 93390. Adrenal cortex 8 hours after onset of fatal hyperpyrexia, showing "tubular" degeneration and depletion of lipoid.  $\times 90$ . Neg. 103712.
- FIG. 23. Case 13, A.I.P. acc. 96871. The adrenal cortex exhibits "tubular" degeneration; cortical lipoid is depleted.  $\times 125$ . Neg. 103912.

20



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Pathology of Hyperpyrexia

PLATE 171

- FIG. 24. Case 4, A.I.P. acc. 82251. Seminiferous tubules 8½ hours after hyperpyrexia. Spermatogenesis is reduced. A giant cell form is visible within the wall of the tubule at the center of the field.  $\times 187$ . Neg. 103733.
- FIG. 25. Case 5, A.I.P. acc. 125672. Seminiferous tubule 11 hours after hyperpyrexia. Spermatogenesis is greatly reduced. Four giant cell forms are present within the lumen.  $\times 275$ . Neg. 103717.
- FIG. 26. Case 15, A.I.P. acc. 89594. Seminiferous tubule 100 hours after hyperpyrexia. Spermatogenesis past the stage of primary spermatogonia has ceased. The latter are greatly reduced in number. Of note is the intraluminal giant cell, the nuclei of which have the same characteristics as the adjacent spermatogonia.  $\times 235$ . Neg. 103703.

# THE ISOLATION OF MUMPS VIRUS AT AUTOPSY\*

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In 1934 Johnson and Goodpasture<sup>1</sup> demonstrated that mumps was caused by a filterable virus and showed that the rhesus monkey could be infected experimentally. Their observations and those of subsequent workers on the pathologic changes in the parotid gland of the monkey provided the first opportunity for the specific correlation of the etiologic agent with these changes. To date, similarly authenticated observations have been lacking on human material. The development by Enders and Cohen<sup>2</sup> of a specific serologic test for mumps, and the propagation of mumps virus in the developing egg by Habel<sup>3</sup> and Levens and Enders<sup>4</sup> have greatly stimulated clinical and laboratory studies on this entity. Using these newly developed technics, information has been obtained on the distribution of the virus in certain body fluids and secretions.<sup>5-7</sup> An opportunity to obtain further information on this subject was afforded when a patient with Sturge-Weber syndrome and pneumonia died with a concurrent mumps infection. The present paper reports the isolation of mumps virus from the tissues of this patient, a description of the specific pathologic changes thus authenticated, and the general pathologic findings.

## *Clinical History and Course*

E. F., a 9-year-old female, had been studied at the Children's Hospital since the age of 7½ months because of frequent psychomotor and grand mal attacks. The presence of a hemangioma involving the left periorbital region and the demonstration of cerebral calcification suggested the diagnosis of Sturge-Weber syndrome.

Approximately 70 hours before death, the patient awoke at night complaining of soreness in the preauricular area. The following morning her mother thought she had localized preauricular swelling and kept her home from school. At noon, the patient complained of malaise and shortly thereafter had a brief grand mal attack. A second grand mal attack, which lasted several hours and was followed by deep coma, occurred approximately 50 hours before death. Thirty-six hours before death, she was admitted to the Children's Hospital. On admission, the patient was comatose and did not respond to painful stimuli. (Temperature, 103° F.; pulse, 74; respirations, 40; blood pressure, 110/65 mm. of Hg.) A large hemangioma was present on the left cheek and forehead. The left pupil was larger than the right and did not react to light. The fundi were normal except for tortuosity of the vessels around the left disk. No definite parotid enlargement was observed. The corneal and pharyngeal reflexes were absent. The lungs were normal to percussion and auscultation; examination of the heart revealed no significant findings. The deep tendon reflexes were hypoactive and Babinski's and Oppenheim's signs were positive bilaterally.

\* Aided in part by a grant from the National Foundation for Infantile Paralysis, Inc.  
Received for publication, September 1, 1948.



Lumbar puncture on admission showed a pressure of 130 mm. of water and yielded clear fluid containing 256 red blood cells per cmm. and no white cells; the protein was 24 mg. per cent, the sugar was slightly decreased by qualitative test, and there was no growth on culture. Blood studies revealed a white blood count of 13,800 with 85 per cent polymorphonuclear cells, 11 per cent lymphocytes, and 4 per cent monocytes. The blood nonprotein nitrogen was 103 mg. per cent and the sugar, 35 mg. per cent. Blood cultures on admission yielded no growth.

A constant intravenous drip of 10 per cent glucose in water was started, followed by normal saline solution; 30,000 units of penicillin was administered every 4 hours. At the end of the first 24 hours in the hospital the blood sugar was 112 mg. per cent and the lumbar puncture yielded fluid containing 139 red cells and 10 white cells per cmm. of which 9 were mononuclear. Swelling of both parotid regions had become apparent. The patient remained unconscious and again began to have intermittent generalized clonic convulsions. Sodium phenobarbital, 0.195 gm., was given intravenously. An hour later the child appeared cyanotic, the heart sounds became inaudible, and the respirations ceased shortly thereafter. During the 36-hour hospital stay the temperature remained between 102.2° and 105.4°F. A third lumbar puncture done 5 hours before death yielded clear fluid with 180 red and 5 white cells per cmm.

### *Post-mortem Findings*

Post-mortem examination (A48-54) was performed 9 hours after death. In the following description only the significant findings are included; the encephalotrigeminal angiomatous process is not described in detail in the present report.

*Gross Description.* Gross examination revealed a purple, sharply delineated, flat, vascular nevus involving the left forehead and upper eyelid. The preauricular regions were not swollen. The cut surfaces of the lungs were moist, but no marked edema was present. The pancreas showed no gross abnormality. On sectioning, the liver revealed a striking yellowish brown coloration in the periphery of the lobules with a darker purple in the central areas; the latter were slightly depressed. The ovaries showed no evidence of hemorrhage or injection; their external surfaces were smooth. The right parotid gland, only, was exposed and sectioned; it did not appear edematous nor were any petechiae seen. The cranium was not thickened and did not display an abnormal vascular pattern. The dura was normal. On the right side the cerebral veins were not remarkable. The left cerebral hemisphere was smaller than the right, and this difference became progressively more marked toward the occipital pole. The entire inferior lateral aspect of the left parietal and occipital hemispheres was overlaid by a thin, dark-red, membranous covering; this on close examination was seen to be made up of a mattress of interlacing meningeal vessels which obscured the convolutions and the sulci. The basal ganglia, the brain stem, and cerebellum were normal. No areas of hemorrhage or infarction were noted in the brain.

*Microscopic Description.* Tissues were fixed in Zenker's acetic acid

solution or 10 per cent neutral formalin and were stained routinely with hematoxylin and eosin; other special stains were employed as noted below.

In sections from all portions of the lungs there was an acute lobular pneumonia, the majority of the alveoli being filled with a polymorphonuclear exudate. The bronchioles contained moderate amounts of purulent material but the distribution of the inflammatory exudate was not bronchopneumonic in pattern. With the Wolbach modification of the Giemsa and the Lerner-Gram\* stains only occasional bacteria were found; these occurred both intracellularly and extracellularly. The spleen contained a few reaction centers in the malpighian corpuscles. The pancreas showed no degenerative or inflammatory alteration. The liver was the seat of an unusual and marked degenerative change. The general architecture of the liver cords remained and no collapse of reticulum was noted after use of Foot's reticulum stain. The parenchymal cells, except in the extreme periphery of the hepatic lobules, stained bright red with eosin, although the Kupffer cells retained the basic dyes. The nuclei of the liver cells stained poorly or not at all, but there was no disintegration of the cell membranes. The parenchymal cells throughout the lobules were filled with fat (scharlach R stain) and this was especially marked in the central areas. No inflammatory cell invasion or evidence of regeneration was observed. The distal convoluted and collecting tubules of the kidney contained numerous hyaline casts; the proximal tubular cells were swollen, many having an increased amount of stainable lipids. The ovary was slightly congested and there was an occasional lymphocyte in the central stroma.

The general architecture of the parotid gland (Figs. 1 and 2) was well preserved, the major alterations in structure occurring in and about the ducts which showed widespread involvement. In the least affected ducts, there was a slight swelling of the epithelial lining cells with an increased granularity of their cytoplasm. The lumina contained a small amount of debris consisting of nuclear fragments, polymorphonuclear leukocytes, and desquamated epithelial cells. In the more severely involved ducts, a large portion of the ductal epithelium had desquamated, leaving a bare basement membrane. Other epithelial cells were considerably swollen and their nuclei pushed to the side of the cell. A rare basophilic, irregularly outlined, cytoplasmic inclusion body could be seen; these filled one-third to two-thirds of the total cell volume. In the most severely involved region, there was great dilatation of the ducts; all

of the epithelial cells were desquamated and a large mass of nuclear and cellular debris choked the lumen. The inflammatory reaction spread for a short distance out into the periductal connective tissues and tangentially involved the adjacent acini. All of the acini at a distance from the ductal structures were completely normal. There was a minor inflammatory reaction in perineurium in one area of the capsular structure; it consisted of a few lymphocytes and monocytes. The interstitial exudate contained little or no fibrin, yet the periductal edema was moderate to marked in degree. In the periductal regions, the lymphocyte was the predominant inflammatory cell; a few large mononuclear and rare polymorphonuclear cells were present. The difference in the inflammatory cell types without and within the ducts was striking.

The brain away from the angiomatous areas appeared normal. No congestion, edema, or hemorrhage, nor inflammatory or degenerative change was seen.

## SUMMARY OF VIRUS ISOLATION AND SEROLOGIC STUDIES

### *Materials Available and Method of Storage*

*Blood.* One sample of blood was obtained 36 hours before death. This was kept at 5°C. for 48 hours and then the serum was separated and stored at -25°C.

*Cerebrospinal Fluid.* Two specimens of cerebrospinal fluid were obtained, consisting of sample A collected 36 hours before death, and sample B collected at autopsy 9 hours after death. Both specimens were kept at 5°C. for 48 hours and then placed in the CO<sub>2</sub> (dry ice) box in sealed glass ampules.

*Tissues.* The following specimens were obtained at autopsy: 5 gm. of small intestine; 1.5 gm. of the upper pole of the right parotid gland; 1.5 gm. of spinal cord from the thoracic region; 6.4 gm. of cerebral cortex from the right parietal area; 3.5 gm. of spleen; and one-half of each ovary, total weight 1.5 gm. The segment of small intestine was opened and washed in two changes of normal saline solution. Individual 10 per cent suspensions by weight of the various tissues were then prepared in isotonic phosphate buffer (pH 7.2) by grinding with alundum. To 9.4 cc. of each organ suspension, 0.6 cc. of a solution of penicillin and streptomycin were added so that each 1 cc. of the resulting mixture contained 500 units of penicillin G and 0.5 mg. of streptomycin base. The suspensions were then centrifuged for 5 minutes at 1000 r.p.m.; the supernatants were removed, sealed in glass ampules, and stored in the CO<sub>2</sub> ice box.

*Isolation and Serologic Identification of an Agent from the  
Parotid Gland*

After storage for 2 days, 0.1 cc. of the 10 per cent parotid gland suspension was inoculated intra-amniotically, as previously described,<sup>8</sup> into 5-day old embryonated hen's eggs. The amniotic fluids were harvested from seven eggs after 6 days' incubation at 35°C.; four of the seven fluids contained hemagglutinins for hen's cells in a titer of 1:8 or greater when tested by a modified Salk technic.<sup>8</sup> A second amniotic passage was done and the average hemagglutinin titer of the fluids when harvested 5 days later was found to be 1:512. Identification studies on the hemagglutinating agent were carried out, employing pooled amniotic fluid from the third egg passage, hereafter termed the EF strain. Complement-fixation tests were done by the technic employed in this laboratory.<sup>8</sup> In these tests, the EF strain and amniotic fluid harvested from the 42nd egg passage of a stock strain of mumps virus were employed as antigens. The serums used included specimens collected during the acute and convalescent phases of mumps from experimentally infected monkeys, from human cases of mumps, and that from patient EF obtained 36 hours before death. The data are presented in Table I. Comparable results were obtained with the two antigens, although there was some variation in titers.

TABLE I

*Results of Complement-Fixation Tests Employing the EF and Stock Strain of Mumps Virus*

Antigen	Serum	Reciprocal of initial dilution of serum					
		8	32	128	512	2048	8192
EF	Acute monkey 148	2	tr	0	nd	nd	nd
Stock	Acute monkey 148	0	0	0	nd	nd	nd
EF	Convalescent monkey 1390	4	4	4	tr	tr	tr
Stock	Convalescent monkey 1390	4	4	4	tr	tr	tr
EF	Acute human 132	4	3	2	tr	nd	nd
Stock	Acute human 132	4	1	tr	0	nd	nd
EF	Convalescent human 132	4	4	4	4	1	tr
Stock	Convalescent human 132	4	4	4	3	tr	0
EF	Acute human 134	nd	4	3	tr	nd	nd
Stock	Acute human 134	3	1	tr	tr	nd	nd
EF	Convalescent human 145	4	4	4	2	tr	tr
Stock	Convalescent human 145	4	4	4	4	4	tr
EF	Patient EF	4	4	2	tr	tr	0
Stock	Patient EF	2	tr	tr	0	0	0

4 = complete fixation of complement.

tr = minimal detectable fixation of complement.

nd = not done.

Agglutinin-inhibition tests were similarly carried out employing the same antigens as above and comparable serums. The technic used was a further modification of the Salk method, which increases the specificity

of the test.<sup>8,9</sup> Two hemagglutinating units of virus were used and the virus-serum mixtures maintained overnight at 5°C. prior to the addition of hen's cells. The results of the tests are summarized in Table II. From all of these observations it is apparent that the agent isolated from the parotid gland behaves as does mumps virus in respect to its growth in eggs, and its hemagglutinative and complement-fixing properties.

#### *Titration of the Egg Infectivity of the Parotid Gland*

Titration of the infectivity of the parotid gland was carried out after storage for 21 days by inoculating groups of eight eggs with falling dilutions from  $10^{-1}$  to  $10^{-6}$  of the suspension made up in a buffer. The amniotic fluids were harvested and tested individually for hemagglutinins after 6 days' incubation. The fluid from one of seven surviving eggs inoculated with the lowest dilution gave a positive test, while the eggs inoculated with higher dilutions were negative. (On subsequent passage of the material from the single positive egg, the agent exhibited the properties of mumps virus.) These findings indicate that the infective titer of the parotid gland suspension was low, although the possibility cannot be eliminated that inactivation of an unknown proportion of the virus occurred during the period of storage.

#### *Isolation of Mumps Virus from Other Organs*

Attempts were made to isolate a virus from the other organ suspensions after they had been stored for 12 to 25 days. Spleen, intestine, spinal cord, brain, and the two specimens of spinal fluid were each inoculated intra-amniotically into eggs and carried for 3 or 4 passages; no hemagglutinating agent was isolated. The suspensions of pancreas and ovary were handled similarly; on the second egg passage, mumps virus was isolated from one of these, but owing to a laboratory error it was impossible to ascertain from which of the two organs the virus had been obtained. Subsequently, three additional attempts to isolate the virus from the suspensions of ovary and pancreas as well as additional attempts to isolate a virus from the other organs failed. Parotid gland material removed from infected monkeys at an appropriate stage of the disease has proved to be a satisfactory complement-fixing antigen.<sup>2</sup> Therefore, search was made for the presence of a similar antigen in suspensions of the parotid gland, spleen, and pancreas from the present case; no specific fixation of complement could be detected.

#### DISCUSSION

With the development of technics for the isolation of mumps virus in eggs, it has been demonstrated that the virus can be isolated with rela-

TABLE II  
Result of Agglutinin-Inhibition Tests Employing the EF and Stock Strain of Mumps Virus

Antigen	Serum	Reciprocal of final dilution of serum													
		8	16	32	64	128	256	512	1024	2048	4096	8192	16384	32768	65536
EF strain	Acute monkey 2	—	—	±A	±A	±A	±A	±A	+	+	+	+	+	+	+
EF strain	Convalescent monkey 2	—	—	—	—	—	—	—	+	+	+	+	+	+	+
EF strain	Acute monkey 1390	—	—	—	—	—	—	—	+	+	+	+	+	+	+
EF strain	Convalescent monkey 1390	—	—	—	—	—	—	—	+	+	+	+	+	+	+
Stock mumps	Acute monkey 1390	—	—	—	—	—	—	—	+	+	+	+	+	+	+
Stock mumps	Convalescent monkey 1390	—	—	—	—	—	—	—	+	+	+	+	+	+	+
EF strain	Acute human 118	—	—	—	—	—	—	—	+	+	+	+	+	+	+
EF strain	Convalescent human 118	—	—	—	—	—	—	—	+	+	+	+	+	+	+
Stock mumps	Acute human 118	—	—	—	—	—	—	—	+	+	+	+	+	+	+
Stock mumps	Convalescent human 118	—	—	—	—	—	—	—	+	+	+	+	+	+	+

+ = Complete agglutination of red blood cells.

+ = Complete agglutination of red blood cells.

± = Incomplete agglutination of red blood cells.

— = No agglutination of red blood cells.

A = Atypical pattern of agglutinated cells.

tive ease directly from the saliva of man during the first few days of the acute illness.<sup>5</sup> Virus has also been isolated in eggs from parotid material of the experimentally infected monkey,<sup>3,4</sup> and the pathologic changes in experimentally infected monkeys have been described.<sup>10-12</sup> As far as is known, mumps virus has not been isolated heretofore from the human parotid gland. While a few descriptions of the pathologic changes in the human parotid gland have appeared in the literature, in no case has the diagnosis been established on other than clinical grounds.

Previous reports on the gross lesions of the human parotid gland in mumps have described enlargement of the gland with edema, congestion, and scattered petechial hemorrhages,<sup>13-16</sup> and similar alterations have been observed in the monkey. In the material obtained from the present case no gross changes were apparent.

The microscopic findings in the parotid gland of the present case consisted essentially of periductal interstitial edema, degeneration of the ductal epithelium with subsequent polymorphonuclear infiltration, and a mononuclear response in the interstitial tis-

sues. These changes in general correspond closely to those previously described in man,<sup>17-20</sup> as well as the monkey, and are therefore confirmatory of the earlier descriptions. However, the extensive acinar degenerative changes observed in the experimental animal<sup>10</sup> and by de Lavergne *et al.*<sup>18</sup> in man were not seen in the present case.

The recent isolation of mumps virus from the spinal fluid<sup>6,7</sup> and the blood<sup>7</sup> in patients with mumps demonstrates that, on occasion at least, the virus has a generalized distribution in the body. In the present case, although there was no specific gross or microscopic evidence of involvement of organs other than the parotid gland, virus was isolated from either the pancreas or the ovary. It appears doubtful that the acute necrotic process in the liver was specifically related to the mumps virus, since it could be explained on the basis of the extensive pneumonia and associated anoxemia; however, there is one somewhat questionable report in the literature of acute hepatic degeneration associated with mumps.<sup>21</sup> Unfortunately, no liver tissue was saved for virus isolation studies. No pathologic or experimental evidence of involvement of the central nervous system by the virus was obtained in this case; this aspect is reviewed by Donohue.<sup>10</sup>

### SUMMARY

An agent, shown to be similar to a known strain of mumps virus in its biologic and immunologic properties, was isolated at autopsy from the tissues of a patient with the Sturge-Weber syndrome, pneumonia, and mumps infection. The virus was found to be present in comparatively low concentration in suspensions of the parotid gland. It was also isolated from either the pancreas or the ovary. No virus could be demonstrated in specimens of spinal fluid.

Pathologic changes which could be attributed to the effect of the mumps virus were limited to the parotid gland and consisted of periductal interstitial edema, a mononuclear response in the interstitial tissues, and degeneration of the ductal epithelium with a polymorphonuclear infiltration. These changes are similar to those previously described in mumps parotitis in man and in the experimentally infected monkey.

We are indebted to Alice H. Northrop for technical assistance and to John Carabitses for the photomicrographs.

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[ Illustrations follow ]



## DESCRIPTION OF PLATE

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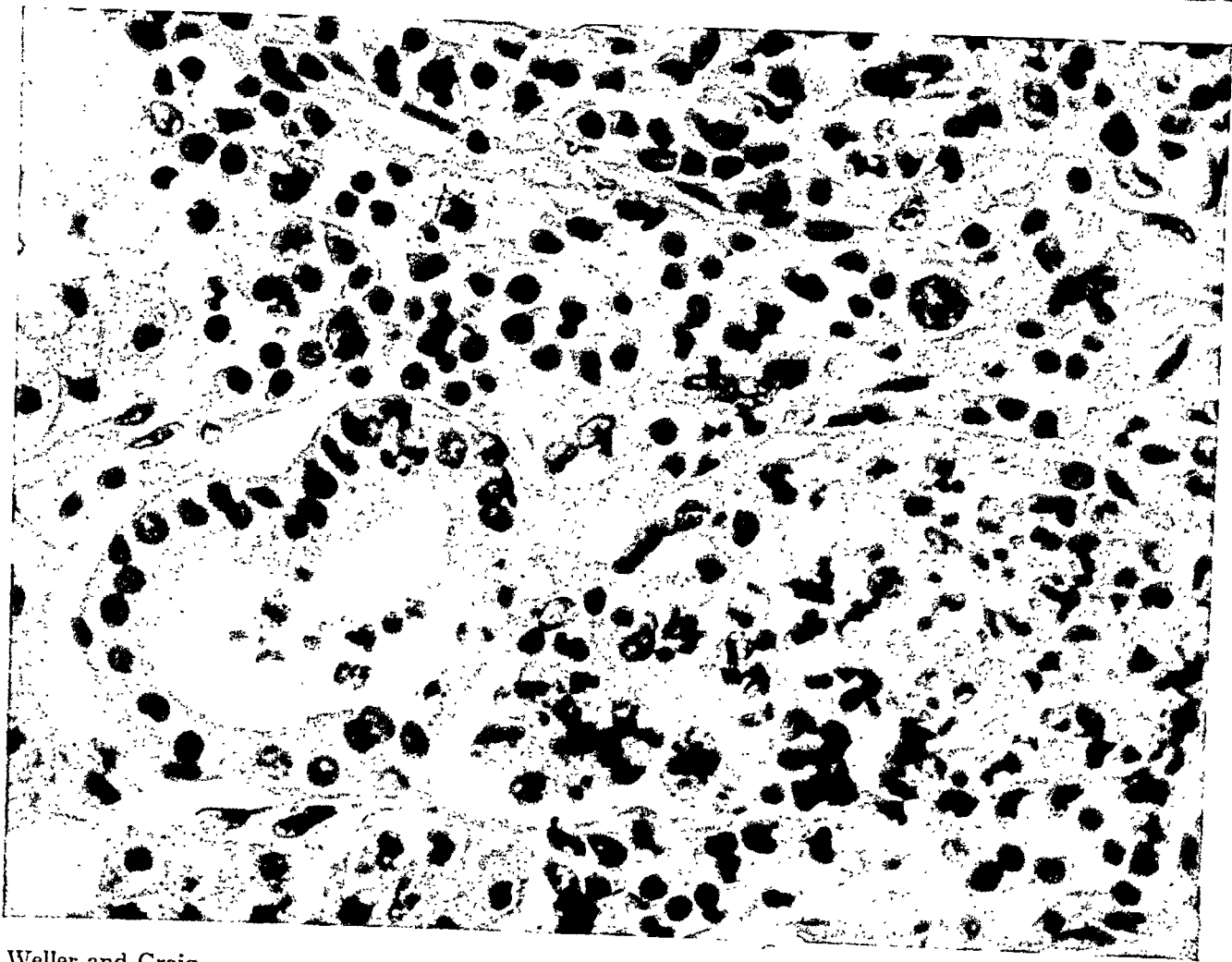
### PLATE 176

- FIG. 1. Parotid gland showing ductal and interstitial involvement. Wolbach's modification of the Giemsa stain.  $\times 175$ .
- FIG. 2. Higher magnification of an area in the parotid gland showing the polymorphonuclear cellular response in the lumen of a duct and the mononuclear infiltration around the duct. Wolbach's modification of the Giemsa stain.  $\times 500$ .

1



2



Weller and Craig



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## THE NATURAL HISTORY OF THE PIGMENTED NEVUS; FACTORS OF AGE AND ANATOMIC LOCATION\*

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The pigmented nevus undergoes a peculiar pattern of development and structural differentiation throughout life. This was first described by Unna<sup>1,2</sup> and little has been added to his original observations. Although different types of pigmented nevi are distinguished, many actually represent the same process in different phases of development.

Recently, stress has been laid on the "junctional" or "dermo-epidermal" nevus, in which there is proliferation of cells in the epidermis and at the dermo-epidermal junction. Traub and Keil,<sup>3</sup> in agreement with Kaufmann-Wolf,<sup>4</sup> regard this proliferation as "potentially malignant (precancerous)" whether it arises early or late in life. To Allen<sup>5</sup> it is the "most precarious of the precancerous lesions" of the skin. Allen,<sup>6</sup> however, points out that this special significance is to be attached only after puberty. Sachs, MacKee, Schwartz, and Pierson<sup>7</sup> distinguish the junctional nevus from the "intradermic nevus" or common mole, and consider it to be the only forerunner of nevocarcinoma. Miescher<sup>8</sup> does not agree with Kaufmann-Wolf that proliferation of cells at the junctional zone is in itself evidence of malignant neoplasia. He regards it as part of "nevogenesis" and precancerous only in the sense that the primary lesion of melanoblastoma<sup>†</sup> is an alteration of this form of proliferation. Some of these authors present objective evidence in support of their views, but more is needed.

The full significance of the histologic features of nevi and melanoblastoma can be learned only after certain data are gathered and cor-

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† Throughout this paper the term melanoblastoma designates the malignant neoplasm for which nevocarcinoma, malignant melanoma, and malignant melanoblastoma are considered by some to be acceptable synonyms.—*Editor*.

relations made. First, the usual appearances and extent of variation of ordinary nevi of patients of all ages and in all anatomic sites are to be determined. Second, comparisons are to be made with proved melanoblastomas. Third, it remains to be determined that histologic evaluation affords reliable information as to the eventual outcome. This investigation undertakes the first of these steps.

The older literature on the histologic appearance of nevi in different age groups and the origin of the nevus cell has been well covered by Fox,<sup>9</sup> Fick,<sup>10</sup> Frédéric,<sup>11</sup> Gans,<sup>12</sup> and Riecke.<sup>13</sup> Theories of epithelial, endothelial, connective tissue, and neural origin were proposed. The more systematic analyses of developmental changes throughout life were by Unna<sup>1,2</sup> and other supporters of the theory of epithelial origin of nevus cells.

In presenting his arguments for the neural nature of nevus cells, Masson<sup>14</sup> again pointed out differences between incipient and old nevi. However, his work and that of Feyrter<sup>15</sup> and of John<sup>16</sup> have been concerned largely with cytologic detail, and comparatively few nevi were reported. Among his 10 cases, Feyrter described a nevus of a patient 1 year old. The remainder were in adults. John based most of his impressions of the appearance of young nevi on a case of leukoderma acquisitum centrifugum of short duration, although the patient was an adult. In his other cases the age varied. The youngest age specifically stated was 12 years. The larger series of Miescher and von Albertini<sup>17</sup> and of Montgomery and Kernohan<sup>18</sup> included nevi of subjects of various ages, but no correlation was made between the appearance of the nevi and the age of the patients. The recent article of Spitz<sup>19</sup> contributes valuable information concerning the nevi of childhood but does not pretend to be an inclusive decade-by-decade analysis.

#### METHOD AND RESULTS

Two hundred nevi, considered to be benign both clinically and histologically, were studied. Representation of various ages and locations was sought for, but otherwise the selection was random. When multiple nevi were excised from one patient, each was considered separately. Blue nevi were omitted.

About ten lesions previously designated as pigmented nevi were reclassified as lentigines. They were from patients of all age groups and, although the distinction of lentigo from nevus is not clear, they were not included in the series. They consisted of hyperpigmented epidermis with occasional clear cells scattered along the deeper zone. There were

no well defined intra-epidermal clumps and no nevus cells in the corium. Occasional melanophores were present, however.

The nevi were subdivided as to the age of the patient and as to the following regions: (1) head and neck, exclusive of conjunctiva; (2) trunk, including external genitalia and perineum; (3) shoulders, arms, buttocks, and thighs (designated as the proximal parts of the extremities); (4) forearms, hands, legs, and feet (designated as the distal parts of the extremities).

Routine surgical material was used. Descriptions and opinions are therefore based on the appearance of one or a few planes of section. While serial sections might disclose certain details, as for example sparse foci of junctional proliferation, the method pursued is adequate for comparisons and for the establishment of a trend in a series as large as this.

Clinical records were examined and as many cases as possible were followed for different periods of time. Histories concerning nevi are usually inadequate and some of the moles were removed incidental to other surgical procedures. Data based on the patient's ideas of duration were obtained in 87.5 per cent of cases, concerning growth of nevi in 87.5 per cent, and follow-up from the time of the surgical excision to the time of the analysis was successful in 88.5 per cent.

In 89.7 per cent of cases in which there was information, the nevus was said to have been present from birth or childhood, or in adults for an indefinitely long time. Therefore, the trends presented in the following discussion are to be correlated not only with the age of the patient but also approximately with the duration of the nevus. The exceptions are analyzed in a later section.

Of the 177 cases with follow-up, there was recurrence in 3 cases but there was no subsequent growth nor was there histologic evidence of melanoblastoma. Nine patients died subsequently, one of carcinoma of the stomach, 2 of carcinoma of the breast, one of heart disease, one of pemphigus, one of alcoholism, one was killed in action in World War II, and for 2 the cause of death was unstated. Follow-up was carried out on one case for 1 month, 2 for 5 months, 2 for 6 months, 28 for 1 year, 18 for 2 years, 8 for 3 years, 20 for 4 years, 60 for 5 to 9 years, 23 for 10 to 14 years, and 15 for 15 to 19 years.

Special attention was directed toward size and shape of the nevi, junctional and intra-epidermal cellular proliferation, mitotic figures, distribution of nevus cells, presence of fibrillar and nerve-like elements, pigmentation, multinucleated cells, inflammation, vascular changes, and

miscellaneous observations. Correlation with certain clinical features was made.

*Size and General Configuration.* The nevi were segregated as narrow stratified, broad stratified, and bulky according to the general unmagnified view of the microscopic section. By "narrow stratified" is meant a slender zone of cells in the epidermis or corium lying parallel to the skin surface. Approximately 0.6 mm. was considered the critical width between narrow and broad. By "broad stratified" is meant a similar zone of cells exceeding 0.6 mm. but not exceeding a width of about 2.0 mm. By "bulky" is meant still broader stratified nevi and large nevi of nodular and irregular shapes. The tabulation of results by age and anatomic location is shown in Table I.

It is evident that there are certain relationships between both age and location and the size and shape of nevi. The narrow stratified nevi are

TABLE I  
*Size and General Configuration of Nevi According to Age and Anatomic Site*

	Age group (years)							Total
	0-9	10-19	20-29	30-39	40-49	50-59	60-	
Head and Neck								
Narrow stratified	2	2	0	0	0	0	1	5
Broad stratified	5	2	1	0	2	0	0	10
Bulky	2	6	7	8	6	11	8	48
Trunk								
Narrow stratified	7	2	0	1	1	3	0	14
Broad stratified	1	2	2	0	0	1	2	8
Bulky	2	8	9	6	9	5	6	45
Extremities, proximal								
Narrow stratified	1	0	0	0	0	0	0	1
Broad stratified	1	1	0	0	1	3	0	6
Bulky	0	3	6	4	8	2	1	24
Extremities, distal								
Narrow stratified	5	4	2	5	4	1	0	21
Broad stratified	2	1	3	1	2	0	0	9
Bulky	2	1	2	0	1	3	0	9
Totals								
Narrow stratified	15	8	2	6	5	4	1	41
Broad stratified	9	6	6	1	5	4	2	33
Bulky	6	18	24	18	24	21	15	126
	30	32	32	25	34	29	18	200

most common in the first decade. The proportion of bulky nevi is greater in the second decade and still greater in the third, but thereafter there is little change. This roughly indicates the rate of growth or cellular multiplication of nevi. At all ages the narrow stratified nevi are least common on the head and neck, and most common on the distal parts of the extremities.

*Junctional and Intra-epidermal Cellular Proliferation.* The cells of a pigmented nevus vary in appearance in the epidermis and corium. By "nevus cell" is meant the characteristic cell of the corium. It is described subsequently. The cells of the epidermis and junctional zone are often designated as the "clear cells." The term is not entirely descriptive because the density, amount, and degree of pigmentation of the cytoplasm vary. Furthermore, there is no clear-cut distinction from nevus cells, for transitions in appearance and direct continuity with nevus cells can be seen. In this paper these cells are spoken of as the intra-epidermal cells or junctional cells of the nevus.

The junctional cells usually appear in round clumps within the epidermis or attached to the deep surface of the epidermis. Clumps of cells separated from the epidermis by collagenous fibrils ("snared off") are designated as subjunctional. Undoubtedly some of the clumps so designated could be demonstrated to connect with the epidermis if serial sections were made. However, for the purposes of comparison and charting it was decided that the term junctional proliferation should be restricted to the cells within the epidermis or joined to it at its deep margin.

Infrequently there is blending of broad bands of cells in the superficial corium with those of the epidermis. These cells are not in clumps and often resemble the deeper nevus cells more closely than the intra-epidermal "clear" cells. This appearance was called junctional proliferation also. However, it is almost invariably associated with the usual type of junctional proliferation and its inclusion does not significantly affect the tabulation.

It was apparent from the start that junctional proliferation is a quantitative consideration. The proliferation was classified as marked, moderate, slight, or none. "Marked" means many foci of proliferation; "moderate," occasional; "slight," sparse; "none," that no such change was found, or, at most, a rare focus of proliferation of a few intra-epidermal cells or junction of a few cells in the epidermis and dermis. In some instances isolated "clear" cells were present in the basal layer of the epidermis. Rarely a few were grouped together. This is often associated with hyperpigmentation of the basal layer, and is not classified as junctional proliferation, because there is no proliferation of clumps or extension of such cells downward from the epidermis. These changes are the same as in lentigo.

The observations of junctional proliferation are applied to hair follicles as well as to the surface epithelium. Proliferation of cells in sweat glands is evident only in the nevi of the first decade. The fact that nevi



in this decade are comparatively acellular permits easy distinction of these foci. However, it is reasonably certain that the diminution in subsequent decades is absolute. The observations are recorded in relation to age and location in Table II and in relation to size and shape of

TABLE II  
*Junctional Proliferation According to Age and Site*

	Age group (years)							Total
	0-9	10-19	20-29	30-39	40-49	50-59	60-	
Head and Neck								
Marked	3	0	0	0	0	0	0	3
Moderate	4	2	0	0	0	1	0	7
Slight	1	3	0	1	0	0	0	5
None	1	5	8	7	8	10	9	48
Trunk								
Marked	5	2	0	0	0	0	0	7
Moderate	4	3	2	0	0	1	0	10
Slight	0	5	3	3	0	1	0	12
None	1	2	6	4	10	7	8	38
Extremities, proximal								
Marked	2	0	0	0	0	0	0	2
Moderate	0	3	1	0	0	0	0	4
Slight	0	1	1	1	0	0	0	3
None	0	0	4	3	9	5	1	22
Extremities, distal								
Marked	8	3	3	3	1	0	0	18
Moderate	1	1	0	2	1	0	0	5
Slight	0	1	1	0	3	1	0	6
None	0	1	3	1	2	3	0	10
Totals								
Marked	18	5	3	3	1	0	0	30
Moderate	9	9	3	2	1	2	0	26
Slight	1	10	5	5	3	2	0	26
None	2	8	21	15	29	25	18	118
	30	32	32	25	34	29	18	200

nevi in Table III. The classification of size and shape is the same as that used in Table I. These tables indicate that there is significant relationship of junctional proliferation with age. The proliferation is most frequent and greatest in the first decade and rapidly diminishes in subsequent age groups.

Because of differences, it is convenient to compare the trends of the nevi of the distal parts of the extremities with those of all other locations, as shown in Table IV. Junctional proliferation in nevi of adults is more frequent when the nevi are located on the distal parts of the extremities. In this site, nonetheless, there is a diminution in degree and frequency with age, and of the 11 nevi so located in patients beyond 40 years of age, only 2 showed it to a moderate or marked degree.

There is no abrupt cessation of junctional proliferation at any age. In Table IV, the first 2 decades are subdivided into 5-year periods. This shows that the diminution in frequency begins before puberty and does not stop abruptly.

TABLE III

*Junctional Proliferation According to Age and General Appearance of Nevus*

	Age group (years)							Total
	0-9	10-19	20-29	30-39	40-49	50-59	60-	
Narrow stratified nevus								
Marked	11	5	2	3	0	0	0	21
Moderate	4	1	0	1	1	1	0	8
Slight	0	0	0	0	3	0	0	3
None	0	2	0	2	1	3	1	9
Broad stratified nevus								
Marked	4	0	0	0	1	0	0	5
Moderate	3	0	1	1	0	0	0	5
Slight	1	4	1	0	0	0	0	6
None	1	2	4	0	4	4	2	17
Bulky nevus								
Marked	3	0	1	0	0	0	0	4
Moderate	2	8	2	0	0	1	0	13
Slight	0	6	4	5	0	2	0	17
None	1	4	17	13	24	18	15	92
Totals								
Marked	18	5	3	3	1	0	0	30
Moderate	9	9	3	2	1	2	0	26
Slight	1	10	5	5	3	2	0	26
None	2	8	21	15	29	25	18	118
	30	32	32	25	34	29	18	200

Although the nevi of childhood are of the narrow stratified type, it is seen from Table III that junctional proliferation is not confined to this type. Clumps of intra-epidermal cells are often found in bulky nevi.

TABLE IV

*Comparison of Junctional Proliferation of Nevi of the Distal Parts of the Extremities with Nevi of All Other Sites*

	Age group (years)								
	0-4	5-9	10-14	15-19	20-29	30-39	40-49	50-59	60-
Distal parts of the extremities	100	100	67	No cases	43	83	29	0	No cases
All other sites	86	86	60	25	12	0	0	8	0

The figures express per cent of nevi showing moderate to marked junctional proliferation.

Contrariwise, the presence of a narrow stratified nevus does not necessarily indicate a nevus of childhood or a nevus with junctional proliferation. Some nevi never do become bulky but nevertheless follow the same trend of diminished junctional proliferation with advancing years.

The exceptions to the trend are discussed later. These include the nevi

of adults which have scanty nevus cells but much junctional proliferation and thereby resemble the nevi of childhood, and the infrequent cases in which nevi of childhood lack junctional proliferation.

*Mitotic Figures.* Mitotic figures are rare in pigmented nevi. Miescher<sup>8</sup> commented that they are exceptionally infrequent in the intra-epidermal nests and may be entirely absent. The rarity was borne out by the present investigation.

In sections from the entire series, mitotic figures were identified in only 13 nevi. In 2, there were 2 mitotic figures in each. In 1, there were 5 mitotic figures. Six of the patients were in the first decade of life, 4 in the second, 2 in the third, and 1 in the fifth. In 7 cases there was moderate or marked proliferation in the epidermis or at the dermo-epidermal junction; in 2 there was slight, and in 4 no proliferation. Of the 19 mitotic figures, 5 occurred in the intra-epidermal, junctional, or immediately subjunctional cells. The remainder were in the nevus cells. They were not seen in nevus cells of the fusiform and fibrillar type. Exceptional note is made of a small nevus, said to be of over 10 years' duration, from the dorsum of the hand of a 42-year-old man. It lacked junctional cells and pigmented cells. Five mitotic figures were counted, scattered at random among the nevus cells. There has been no recurrence of this lesion 10 years after removal.

*Distribution of Nevus Cells.* In all cases there were nevus cells in the upper part of the corium. There was a tendency for the cells of nevi of the trunk to be closer to the epidermis than the cells of nevi of the head and neck, which tended to have a definite (although thin and focally interrupted) zone of collagenous connective tissue just beneath the epidermis.

The number of nevi with cells in the mid-corium and deep corium are shown in Table V, correlated with age and site. The nevi were classified as to the presence of none, few, or many nevus cells in the corium deep to the uppermost two or three bands of coarse collagenous tissue. This is the collagenous stratum which can be traced laterally to the adjacent normal corium, and not the finer fibrils intermingled with nevus cells.

Nevus cells were present in the deep parts of the corium in about the same proportion of nevi in all age groups. However, it is true that in older life the mass of the deep cells was greater than in the first decade as was also the mass of the superficial cells. This has not been analyzed statistically but was readily apparent in the histologic material. The largest proportion of nevi with deeply situated cells were of the head and neck. Nevi of the proximal part of the extremities, trunk, and distal part

of the extremities showed a progressively smaller proportion in the order stated.

The nevi with cells deep in the corium were further analyzed as to their relationship to hair follicles and sweat glands. Observations con-

TABLE V  
*Nevi Showing Cells in Deeper Part of Corium*

	Age group (years)							Total
	0-9	10-19	20-29	30-39	40-49	50-59	60-	
Head and neck								
None	0	0	0	0	1	0	0	1
Few	2	2	1	1	1	0	2	9
Many	7	7	7	7	6	8	6	48
Inadequate biopsy	0	1	0	0	0	3	1	5
Trunk								
None	3	4	0	3	1	3	3	17
Few	3	2	8	1	6	4	3	27
Many	3	6	3	1	2	2	0	17
Inadequate biopsy	1	0	0	2	1	0	2	6
Extremities, proximal								
None	0	0	0	0	0	2	0	2
Few	1	3	6	2	3	1	1	17
Many	1	1	0	2	6	2	0	12
Inadequate biopsy	0	0	0	0	0	0	0	0
Extremities, distal								
None	1	4	1	4	3	2	0	15
Few	4	0	3	1	3	1	0	12
Many	3	2	3	1	1	1	0	11
Inadequate biopsy	1	0	0	0	0	0	0	1
Totals								
None	4	8	1	7	5	7	3	35
Few	10	7	18	5	13	6	6	65
Many	14	16	13	11	15	13	6	88
Inadequate biopsy	2	1	0	2	1	3	3	12
	30	32	32	25	34	29	18	200

cerning this were noted by Unna,<sup>1,2</sup> Masson,<sup>14</sup> and others. The deep nevus cells were classified as: perifollicular, if all the cells formed a distinct aggregation about hair follicles and sweat glands; perifollicular and scattered, if aggregates about follicles and sweat glands were accompanied by a more diffuse distribution; scattered, if there was no distinct grouping. The results are presented in Table VI which shows that in a majority of nevi the deep cells tend to have a perifollicular pattern. Even if some of the cells appear diffusely scattered between follicles there is usually a perceptible aggregation about these structures. The perifollicular arrangement is most striking in the nevi of children, in which case the mass of cells is not bulky. It is least conspicuous in the nevi of the head and neck, especially in adult life, and is progressively

more noticeable in the proximal parts of the extremities, the trunk, and the distal parts of the extremities. The paradoxical lack of distinct perifollicular grouping in nevi of the head and neck in adults is difficult to explain but it may be related to the profusion of follicles and sweat glands causing a confluence of aggregates.

TABLE VI  
*Distribution of the Cells in the Deep Corium*

	Age group (years)							Total
	0-9	10-19	20-29	30-39	40-49	50-59	60-	
Head and Neck								
Perifollicular	3	2	0	0	0	0	0	5
Scattered	4	4	7	3	4	5	5	32
Perifollicular and scattered	2	3	1	5	3	3	3	20
Inadequate biopsy	0	1	0	0	0	3	1	5
Trunk								
Perifollicular	3	1	6	1	3	3	1	18
Scattered	1	3	2	1	3	0	2	12
Perifollicular and scattered	2	4	3	0	2	3	0	14
Inadequate biopsy	1	0	0	2	1	0	2	6
Extremities, proximal								
Perifollicular	1	3	4	1	1	1	1	12
Scattered	0	0	0	1	3	1	0	5
Perifollicular and scattered	1	1	2	2	5	1	0	12
Inadequate biopsy	0	0	0	0	0	0	0	0
Extremities, distal								
Perifollicular	5	0	5	1	2	0	0	13
Scattered	1	0	0	0	2	1	0	4
Perifollicular and scattered	1	2	1	1	0	1	0	6
Inadequate biopsy	1	0	0	0	0	0	0	1
Totals								
Perifollicular	12	6	15	3	6	4	2	48
Scattered	6	7	9	5	12	7	7	53
Perifollicular and scattered	6	10	7	8	10	8	3	52
Inadequate biopsy	2	1	0	2	1	3	3	12
	26	24	31	18	29	22	15	165

In general, deep nevus cells are found among and around the follicular epithelial cells and among and around the cells of sweat glands. This in itself does not prove an epithelial origin, for nerve fibrils and terminations are also abundant in these distributions.

Occasionally there are nevus cells among the fibers of the arrectores pilorum muscles. This may appear to refute the epithelial theory of origin but this in turn is not conclusive for the muscle is intimately related with the follicle anatomically and embryologically.

Enclosure of small vascular channels and small nerves occurs especially in the large nevi of adult life. It is not constant in random sections and does not in itself substantiate a theory of either perivascular or perineural origin of nevus cells.

Nevus cells occasionally extend as deeply as the subcutaneous fat. Infiltration among fat cells is most frequent in nevi of the head. In nevi which overlie skeletal muscle, as in those of the lip or eyelid, penetration to and around some of the superficial skeletal muscle fibers may take place. This is not to be interpreted as invasion (Fig. 8).

In summary, the superficial cells of a nevus proliferate in a zone lying parallel to the surface and in their growth tend to separate the coarsely fibrous part of the corium from the epidermis. The deep cells of a nevus tend to be distributed in and about follicles and sweat glands, although diffusely scattered cells are seen also.

*Fibrillar and Nerve-like Elements.* There are transitional appearances between the intra-epidermal "clear" cells and the nevus cells of the corium. The latter in turn undergo progressive changes. In the more superficial corium, especially in nevi of youth, the nevus cells may be exactly the same as the cells at the dermo-epidermal junction. A little more deeply the cells are usually somewhat smaller, usually pigment-free, but retain a large nucleus and nucleolus. Still more deeply, as a rule, the cells are smaller, nuclei are more compact, and pigment is almost always absent. Cells with nucleoli are not so abundant here as in the superficial corium. Occasionally a central pale round spot is seen in the nucleus, larger than a nucleolus and occasionally having a faint brown color. The nevus cells may be spread in large masses, or in groups surrounded by fibrils. There is gradual transition to cells of oval or fusiform type. Definition of cytoplasmic borders is lost and nuclei are adjacent to or enclosed by faintly eosinophilic fibrils, which usually run in the same axis as that of the nucleus and are sometimes arranged as ribbon-like fasciculi. These are usually seen in the lowermost part of the mass of nevus cells but may be both deep and superficial, especially in the nevi of older people. In some the fibrils are abundant and compact and often have a resemblance to Wagner-Meissner corpuscles. In the more fibrillar nevi nuclei are in many instances irregular in shape, densely stained, and often clumped. In the fibrillar parts of a nevus there are comparatively few nuclei. It is not possible to state from this study how much of this is relative, in view of the great increase of fibrils, but an absolute decrease is favored.

That the above transition in the appearance of nevus cells is developmental is shown by correlation of fibrillar elements with age. The presence of fibrils is quantitative. The degree of occurrence was classified as absent or slight, moderate, and marked. The degrees are arbitrary and difficult to define. By absent or slight is meant that none or only a few cells were associated with fibrils. By marked is meant that extensive

areas in the microscopic section were made up of fibrillar cells. Often the areas were comparatively anuclear, composed almost entirely of fibrils alone. Moderate is an intermediate appearance; the fibrillar cells were not predominant nor did they occupy large foci. The results are shown in Table VII.

TABLE VII.  
*The Presence of Fibrillar Elements in Nevi*

	Age group (years)							Total
	0-9	10-19	20-29	30-39	40-49	50-59	60-	
Head and Neck								
None or few	4	4	0	1	0	0	0	9
Moderate number	2	5	4	2	1	0	0	14
Many	3	1	4	5	7	11	9	40
Trunk								
None or few	9	2	2	1	0	1	0	15
Moderate number	1	5	4	0	3	3	1	17
Many	0	5	5	6	7	5	6	34
Inadequate biopsy	0	0	0	0	0	0	1	1
Extremities, proximal								
None or few	1	1	0	0	0	0	0	2
Moderate number	1	2	2	1	1	1	1	9
Many	0	1	4	3	8	4	0	20
Extremities, distal								
None or few	5	4	3	4	2	0	0	18
Moderate number	4	1	3	1	3	1	0	13
Many	0	1	1	1	2	3	0	8
Totals								
None or few	19	11	5	6	2	1	0	44
Moderate number	8	13	13	4	8	5	2	53
Many	3	8	14	15	24	23	15	102
Inadequate biopsy	0	0	0	0	0	0	1	1
	30	32	32	25	34	29	18	200

A further segregation of nevi was made in which the nevi with foci which resembled neurofibrillar tissue were selected. Here again, criteria of selection cannot be sharply defined. It is based on the presence of readily recognized foci of delicate fibrillar tissue. Usually this is arranged in delineated ribbons and nests. In many, there are structures resembling Wagner-Meissner corpuscles. Thus the cylindroid, ribbon-like, and plate-like structures described by Masson<sup>14</sup> are all included. The results are shown in Table VIII.

It is evident that there is a progressive increase of fibrils and what resembles neurofibrillar tissue with increasing age, and that such tissue is the final phase of differentiation of the nevus cells. Broders and Fletcher,<sup>20</sup> in the interpretation of a nevus which had undifferentiated cells superficially and resembled neurofibrillar tissue deeply, came to

the same conclusion. Interpretation of the fibrillar tissue or structures resembling tactile corpuscles as the source of the nevus cells is not justifiable.

It is thought, in agreement with the interpretations of others, notably

TABLE VIII  
*The Presence of Nerve-like Elements*

	Age group (years)							Total
	0-9	10-19	20-29	30-39	40-49	50-59	60-	
Head and neck								
None	9	10	8	5	4	3	2	41
Present	0	0	0	3	4	8	7	22
Trunk								
None	10	12	9	5	6	8	4	54
Present	0	0	2	2	4	1	2	11
Inadequate biopsy	0	0	0	0	0	0	2	2
Extremities, proximal								
None	6	3	6	4	7	2	1	29
Present	0	1	0	0	2	3	0	6
Extremities, distal								
None	5	6	6	6	7	2	0	32
Present	0	0	1	0	0	2	0	3
Totals								
None	30	31	29	20	24	15	7	156
Present	0	1	3	5	10	14	9	42
Inadequate biopsy	0	0	0	0	0	0	2	2
	30	32	32	25	34	29	18	200

Kromayer<sup>21</sup> and Masson,<sup>14</sup> that the fibrils arise from the nevus cells and are not a reactive fibrosis.

In general, nevi in which fibrils are abundant are not associated with much junctional proliferation, but there are exceptions; for example, the instance recorded in the second decade (actually 12 years of age) and one instance in the third decade. In one nevus in a 7-months-old child, located on the back and not part of this series of 200, there was marked junctional proliferation but the major part of the nevus, extending from the upper cutis into the subcutaneous fat, consisted of fibrillar cells.

*Pigmentation.* The degree of pigmentation was arbitrarily and roughly expressed as none, 1 plus, 2 plus, 3 plus, and 4 plus. Pigmentation of the basal epidermis was not included even though it was intense in some instances and resembled the changes of lentigo. On the other hand, pigment in chromatophores among the nevus cells was included. The results in Table IX show the trend as to age and anatomic site. This



indicates that pigmentation tends to diminish with age. Furthermore, it tends to be least intense in nevi of the head and neck. The latter observation may be correlated with the additional observation that pigment is largely related to nevus cells lying in a stratum just beneath the epidermis and is not common in nevus cells around the follicles and

TABLE IX  
*Pigmentation According to Age and Site*

	Age group (years)							Total
	0-9	10-19	20-29	30-39	40-49	50-59	60-	
Head and neck								
None	1	0	0	3	2	4	3	13
One plus	3	4	5	5	3	4	3	27
Two plus	2	6	2	0	3	2	0	15
Three plus	1	0	1	0	0	0	0	2
Four plus	2	0	0	0	0	1	3	6
Trunk								
None	0	0	0	1	3	1	3	8
One plus	1	1	3	3	5	5	4	22
Two plus	4	6	4	1	2	3	1	21
Three plus	4	3	4	2	0	0	0	13
Four plus	1	2	0	0	0	0	0	3
Extremities, proximal								
None	0	0	0	1	2	3	1	7
One plus	0	0	2	2	3	1	0	8
Two plus	0	2	4	1	4	0	0	11
Three plus	2	0	0	0	0	1	0	3
Four plus	0	2	0	0	0	0	0	2
Extremities, distal								
None	0	0	0	1	1	1	0	3
One plus	1	1	3	2	1	1	0	9
Two plus	0	2	3	2	1	2	0	10
Three plus	7	3	1	1	4	0	0	16
Four plus	1	0	0	0	0	0	0	1
Totals								
None	1	0	0	6	8	9	7	31
One plus	5	6	13	12	12	11	7	66
Two plus	6	16	13	4	10	7	1	57
Three plus	14	6	6	3	4	1	0	34
Four plus	4	4	0	0	0	1	3	12
	30	32	32	25	34	29	18	200

sweat glands or diffusely scattered in the deeper corium. Even in the nevi of childhood in which intra-epidermal clumps of "clear" cells are seen along follicles and sweat glands, pigment is scanty or absent in these locations. The nevi of the face and neck, as previously noted, contain relatively more of the deeply placed diffuse and perifollicular cells.

The age distribution of pigmentation suggests a relationship to junctional proliferation, and this is borne out in the correlation of pigmentation and junctional proliferation, as shown in Table X.

The cells at the dermo-epidermal junction and of the upper part of the corium tend to be the most intensely pigmented and hence there is a rough correlation with proliferation at the dermo-epidermal junction. As pointed out, the junctional and subjunctional cells are scanty or absent in older age groups. The presence of chromatophores, usually in

TABLE X  
*Relationship of Pigmentation and Junctional Proliferation*

	Age group (years)							Total
	0-9	10-19	20-29	30-39	40-49	50-59	60-	
No junctional proliferation								
No pigment	0	0	0	6	8	9	7	30
One plus	2	4	11	7	11	11	7	53
Two plus	0	3	7	1	9	3	1	24
Three plus	0	1	3	1	1	1	0	7
Four plus	0	0	0	0	0	1	3	4
Slight junctional proliferation								
No pigment	0	0	0	0	0	0	0	0
One plus	1	2	1	3	0	0	0	7
Two plus	0	6	3	1	1	2	0	13
Three plus	0	1	1	1	2	0	0	5
Four plus	0	1	0	0	0	0	0	1
Moderate junctional proliferation								
No pigment	0	0	0	0	0	0	0	0
One plus	1	0	0	2	1	0	0	4
Two plus	3	5	1	0	0	2	0	11
Three plus	3	1	2	0	0	0	0	6
Four plus	2	3	0	0	0	0	0	5
Marked junctional proliferation								
No pigment	1	0	0	0	0	0	0	1
One plus	1	0	1	0	0	0	0	2
Two plus	3	2	2	2	0	0	0	9
Three plus	11	3	0	1	1	0	0	16
Four plus	2	0	0	0	0	0	0	2
Totals								
No pigment	1	0	0	6	8	9	7	31
One plus	5	6	13	12	12	11	7	66
Two plus	6	16	13	4	10	7	1	57
Three plus	14	6	6	3	4	1	0	34
Four plus	4	4	0	0	0	1	3	12
	30	32	32	25	34	29	18	200

the superficial part of the nevus, suggests that pigment is discharged from cells but actual death and phagocytosis of pigmented nevus cells would produce the same effect. Whether the deeper cells have lost, or never had, the property of pigment production in these sites cannot be answered from the above observations.

*Multinucleated Cells.* Multinucleated cells are common in nevi. Occasionally their presence or absence is carefully noted in the differentia-

tion of a nevus from melanoblastoma (Becker,<sup>22</sup> Dawson<sup>23</sup>), but the reader does not gain a clear idea of their special significance and is left with the impression that they indicate melanoblastoma. Spitz,<sup>19</sup> however, stressed the importance of the presence of giant cells in "juvenile melanoma," which distinguished them from "adult melanoma" in about half the cases.

It was found that multinucleated cells are of two types. The first appears as a comparatively small cluster of nuclei. In some instances these appear to lie in a common cytoplasm. In others this is not certain and the multinucleated cell may actually be an aggregate of cells, distinguished from other aggregates only by the small circular pattern of the nuclear cluster. Frequently the nuclei are densely stained, vacuolated, or distorted, appearing in curved or irregular shapes. A multinucleated cell of the second type is large and the cytoplasm is distinct. Usually the nuclei are in a peripheral ring but may be arranged in central clusters. Nuclei are not pyknotic. The chromatin pattern is delicate and sometimes there is a nucleolus. These giant cells are usually in the superficial part of the nevus.

The occurrence of multinucleated cells of both types, according to age and location, is seen in Table XI. There is no significant change in the

TABLE XI  
*Giant Cells of Both Kinds*

	Age group (years)							Total
	0-9	10-19	20-29	30-39	40-49	50-59	60-	
Head and neck								
None	4	6	1	2	5	4	4	26
Scarce	1	2	1	2	2	1	2	11
Many	4	2	6	4	1	6	3	26
Trunk								
None	13	6	3	5	6	6	3	42
Scarce	0	4	7	1	3	2	4	21
Many	1	2	1	1	1	1	1	8
Extremities, proximal								
None	2	2	3	3	3	3	1	17
Scarce	0	1	2	0	4	1	0	8
Many	0	1	1	1	2	1	0	6
Extremities, distal								
None	3	5	4	6	6	1	0	25
Scarce	2	1	2	0	1	3	0	9
Many	0	0	1	0	0	0	0	1
Totals								
None	22	19	11	16	20	14	8	110
Scarce	3	8	12	3	10	7	6	49
Many	5	5	9	6	4	8	4	41
	30	32	32	25	34	29	18	200

incidence throughout life. They are most abundant in nevi of the head and neck, and least in the nevi of the distal parts of the extremities.

The occurrence of multinucleated cells of the second type, according to age and location, is seen in Table XII. Here again, there is no sig-

TABLE XII  
*Large Giant Cells*

	Age group (years)							Total
	0-9	10-19	20-29	30-39	40-49	50-59	60-	
Head and neck								
None	7	8	5	7	6	9	8	50
Scarce	1	1	2	1	2	1	0	8
Many	1	1	1	0	0	1	1	5
Trunk								
None	14	8	7	5	8	9	8	59
Scarce	0	3	4	2	1	0	0	10
Many	0	1	0	0	1	0	0	2
Extremities, proximal								
None	2	3	5	4	5	4	1	24
Scarce	0	0	1	0	3	0	0	4
Many	0	1	0	0	1	1	0	3
Extremities, distal								
None	4	5	6	6	7	4	0	32
Scarce	1	1	1	0	0	0	0	3
Many	0	0	0	0	0	0	0	0
Totals								
None	27	24	23	22	26	26	17	165
Scarce	2	5	8	3	6	1	0	25
Many	1	3	1	0	2	2	1	10
	30	32	32	25	34	29	18	200

nificant change throughout life. The cells are least common in nevi of the distal parts of the extremities and most common in nevi of the head and neck, and proximal parts of the extremities. It is to be expected that giant cells of both types would be less numerous in nevi of the distal parts of the extremities because these are usually "narrow stratified" nevi and cells of all types are fewer in them than in the bulky nevi of other sites.

*Inflammation.* The nevi were studied as to the degree of inflammation present in and around each of them. This was arbitrarily subdivided as none or questionable, slight, moderate, and marked. Almost every inflammatory lesion was of chronic or subacute nature. In 4 cases the inflammation was acute exudative. In 2 of these there was ulceration. For simplification, all types are grouped and differentiated only as to degree. The results are shown in Table XIII.

It is seen that inflammation is more common in the older age groups

and is infrequent in the first decade. There is therefore no parallelism between inflammation and active (benign) proliferation of a nevus. Inflammation was more frequent and more active in the larger nevi and especially in those with large hair follicles. The follicles in nevi are often

TABLE XIII  
*Inflammation*

	Age group (years)							Total
	0-9	10-19	20-29	30-39	40-49	50-59	60-	
Head and neck								
None	8	4	3	3	3	1	2	24
Slight	0	2	3	3	4	5	3	20
Moderate	1	4	2	2	1	4	4	18
Marked	0	0	0	0	0	1	0	1
Trunk								
None	10	4	5	5	5	6	2	37
Slight	0	6	5	2	4	3	4	24
Moderate	0	0	1	0	0	0	2	3
Marked	0	2	0	0	1	0	0	3
Extremities, proximal								
None	2	4	4	0	5	4	1	20
Slight	0	0	1	4	2	1	0	8
Moderate	0	0	0	0	2	0	0	2
Marked	0	0	1	0	0	0	0	1
Extremities, distal								
None	8	5	3	6	5	2	0	29
Slight	0	1	3	0	2	1	0	7
Moderate	1	0	1	0	0	0	0	2
Marked	0	0	0	0	0	1	0	1
Totals								
None	28	17	15	14	18	13	5	110
Slight	0	9	12	9	12	10	7	59
Moderate	2	4	4	2	3	4	6	25
Marked	0	2	1	0	1	2	0	6
	30	32	32	25	34	29	18	200

dilated and filled with keratin debris. There is associated with this in many instances a perifollicular or intrafollicular leukocytic infiltration and exudation. The cells are principally lymphocytes but occasionally eosinophils and neutrophils are present. Occasionally there are foreign body giant cells. Infiltration of lymphocytes, not associated with follicles, is usually most noticeable at the lateral margins of nevi close to the surface of the normal skin. Less frequently, it is at the deep margin. It is difficult to detect slight degrees of lymphocytic infiltration or fibroblastic proliferation, for they are often closely imitated by the nevus cells. Next to lymphocytes, eosinophils were the most common form of leukocyte.

Inflammation may occur in any site. The greatest frequency is in nevi of the head and neck, evidently because of the large number of hair

follicles present. It is least common in nevi of the extremities, especially of the distal parts. This is partly explained by the large percentage of hairless nevi of the distal extremities and by the preponderance of flat ("narrow stratified") nevi which are less subject to trauma. These observations are contrary to widespread beliefs.

Inflammation has been stressed as an important sign of melanoblastoma (Miescher<sup>8</sup>), but from the above it is evident that many other factors must be considered. However, its presence in a flat hairless nevus is more significant than in a large nevus with numerous hair follicles.

*Vascular Changes.* In large nevi, especially of adult life, and nevi which tend to be pedunculated, dilatation of capillaries, venules, and lymphatics is conspicuous. In addition, interstitial edema is noted occasionally. Often the nevus cells are spread apart and lacunae suggest acini or channels. Rarely the faintly staining fluid precipitates in these spaces. Usually the above changes occur in nevi which are at least partly fibrillar. Unna's<sup>1,2</sup> concept that the atrophy of nevus cells and fibril formation ("molluscoid" changes) are directly related to constriction at the base and lymph edema is to be considered. However, fibrillar changes in flat nevi and nonpedunculated nevi with or without vascular dilatation are hardly compatible with this theory. Yet, that trophic disturbance may account for the later changes in nevi cannot be denied.

*Miscellaneous Observations.* Focal ossification in the subcutaneous fat was noted in 2 cases, and focal calcification of the subcutaneous fat in one case.

One nevus of the back of a 10-year-old child had been treated by irradiation. Because of subsequent dermatitis and ulceration the lesion was excised widely. The nevus was benign histologically but the dermatitis was complicated by squamous cell carcinoma. The case was followed for 18 years without recurrence or metastasis.

In the first part of this investigation certain features of nevi were analyzed as they relate to age and anatomic site. In order to facilitate further discussion, a summary of certain trends is advantageous.

Four features were greatly influenced by age: junctional and intra-epidermal cellular proliferation, mitotic figures, size (or total cellularity), and differentiation of nevus cells to fibrillar forms. In order to simplify further discussion, the nevi have been re-evaluated and segregated into five types, determined by the above features with the exception of the presence of mitotic figures. The types are labelled A, B, C, D, and E. The characteristics of these types are:

A. The nevus consists mostly of intra-epidermal cells and cells at the dermo-epidermal junction. This includes, of course, intra-epidermal cells along hair follicles and sweat glands. There are few nevus cells in the corium.

B. The nevus consists of intra-epidermal cells and cells at the dermo-epidermal junction and there are nevus cells in fair number in the corium.

C. The nevus consists mostly of nevus cells in the corium but there is still moderate to marked cellular proliferation in the epidermis or at the dermo-epidermal junction.

D. The nevus consists of nevus cells and there is no, or very little, junctional proliferation.

E. This corresponds to type D except that many of the nevus cells are fibrillar in type.

The general trend in regard to the appearance of nevi at different ages is summarized in Table XIV. It is seen that there is a definite trend from the A type to the E type and that the age of the patient can be correlated roughly with the structure of the nevus. However, there are exceptions and these will be discussed subsequently.

TABLE XIV  
*Distribution of Nevi as to Type at Different Ages*

Age group (years)	Type of nevus					Total
	A	B	C	D	E	
0-4	3	6	3	1	0	13
5-9	3	8	4	2	0	17
10-14	2	2	6	6	0	16
15-19	0	1	2	10	3	16
20-29	1	1	3	13	14	32
30-39	1	2	2	6	14	25
40-49	0	0	2	8	24	34
50-59	0	0	2	6	21	29
60-	0	0	0	2	16	18
Totals	10	20	24	54	92	200

*Appearance of Multiple Nevi.* In those instances in which multiple nevi from the same patient were removed at the same time the microscopic appearance was practically identical. These are analyzed in Table XV. In only one case was there notable variation, in which instance one nevus was of type A and one of type D.

*Duration of Nevi.* Data concerning the patients' ideas of the duration of the nevi were obtained in 87.5 per cent of the cases. Of these, 157 nevi or 89.7 per cent were stated to have been present since birth or childhood, or, in adults, for an indefinitely long period of time. Hence the trend noted above correlates with the duration of the nevus as well as with the age of the patient.

In 18 instances the nevi were stated to be of significantly shorter duration than the age of the patients. These are analyzed in Table XVI.

It may be suspected from the trend described that nevi of shorter duration in adults should resemble the nevi of childhood. However,

TABLE XV  
*Cases of Multiple Nevi Analyzed According to Type*

	Age group (years)						
	0-9	10-19	20-29	30-39	40-49	50-59	60-
Number	3	1	1	3	4	1	1
Combination of types	CAA BB DD	ED	DA	AC EE CB	EE EE ED DC	EE	EE

this conclusion is not substantiated. Most of these nevi correspond in type to those which date from birth or childhood. In many instances the histories are questionable. It does not seem right that a large nevus composed often of fibrillar cells should be present only a matter of

TABLE XVI  
*Analysis of Nevi Stated to Be of Significantly Shorter Duration than Age of Patient*

Case no.	Type of nevus	Age	Location	Reason removed	Stated duration	Recent growth	Follow-up
32	D	18	Behind ear	Growth	2-3 mos.	Yes	7 yrs.
64	D	23	Nose	Unknown	6 mos.	Yes	4 yrs.
70	E	29	Behind ear	Annoying when put glasses on	10 yrs.	Yes	2 yrs.
80	D	27	Breast	Incidental	2 yrs.	Yes	4 yrs.
81	E	27	Perineum	Incidental	6 mos.	No	9 yrs.
97	E	33	Scalp	Interfered with combing of hair	Months	Yes	4 yrs.
98	E	33	Scalp	Interfered with combing of hair	Months	Yes	4 yrs.
105	E	34	Back	Incidental	8 yrs.	None in last 3 yrs.	4 yrs.
115	A	37	Sole	Incidental	1 yr.	Yes*	1 yr.
119	B	36	Sole	Recent appearance	Few days	Yes	1 yr.
126	E	43	Chin	Incidental	4 or 5 yrs.	Yes	3 yrs.
149	D	41	Toe	Irritation	10 yrs.	Yes	6 yrs.
150	C	41	Toe	Irritation	10 yrs.	Yes	6 yrs.
176	E	56	Shoulder	Incidental	2 yrs.	No	12 yrs.
184	E	74	Neck	Growth	6 yrs.	Yes	5 yrs.
185	E	61	Canal of ear	Growth and pain	15 yrs.	Yes	5 yrs.
186	E	65	Face	Growth	1 yr.	Yes	5 yrs.
187	E	60	Eyelid	Growth	5 yrs.	Yes	13 yrs.

\* Classed as recent growth because of recent appearance.

months or a few years, as was stated in some instances. It may be, as is the case in nevi of the scalp, back, auditory canal, behind the ear, or on the perineum, that the patients were not aware of the presence of the nevi for the greater part of their lives. Furthermore, since some were removed incidental to other surgical procedures, the patient may not



have been aware of the nevus at any time. The 3 nevi more in keeping with those of childhood (types A, B, and C) were of the feet.

*Clinical Symptoms in Relation to Location of Nevus and Type of Nevus.* The major clinical manifestations were grouped under the following headings: growth, irritation, incidental, and other manifestations. Historical details in the records usually were scanty and interview with the patient often added little information. In most instances the rate of growth was not specified or was poorly described. It was decided that the major classification should be based on the reason for removal of the nevus. Thus, if accelerated growth were the reason for removal it may be accepted that the growth was recent and appreciable. Recent appearance amnestically is considered evidence of recent growth. By irritation is meant soreness, ulceration, or bleeding. By incidental manifestations are meant such circumstances as removal in the course of other surgical procedures, for cosmetic reasons, or as a precautionary measure to avoid melanoblastoma, or because of location. Some nevi of the feet were removed for no other reason than location. In the group called "other manifestations" there was a variety of reasons, including x-ray dermatitis from previous efforts to destroy the nevus, removal in order to fit on glasses, shoe, or football helmet, and interference with combing the hair.

The reasons for removal were subdivided and compared with age and anatomic site, as shown in Table XVII.

Nevi of childhood were removed more frequently because of growth than nevi of adults. Nevi of the distal parts of the extremities were removed because of growth more often than nevi elsewhere. Nevi of the head and neck were next in this regard. The greatest single category in all anatomic sites is incidental removal. However, this accounts for the great majority of those of the trunk and proximal parts of the extremities but for less than one-half of those of the head and distal parts of the extremities. The frequent incidental removal of nevi during abdominal and perineal operations and during delivery accounts for this. Irritation is about equally important as a complaint in regard to all anatomic sites. It is a common complaint in youth as concerns nevi of the trunk but not so in middle and old age. It is a frequent complaint in reference to nevi of the head and neck in adult life and old age, but this is not true of these nevi in youth.

The histologic appearance of the nevus was compared with the major complaint and other symptoms as shown in Table XVIII.

From the totals of all ages it is construed that growth is characteristic of nevi in types A, B, and C. There is alleged growth in other types

TABLE XVII

*Reasons for Removal of Nevus as Related to Site and Age*

	Age group (years)							Total
	0-9	10-19	20-29	30-39	40-49	50-59	60-	
Head and neck								
Growth	5	2	1	0	0	5	5	18
Irritation	1	0	0	2	1	1	1	6
Incidental	3	7	3	2	6	5	2	28
Other	0	0	0	2	1	0	1	4
Unknown	0	1	4	2	0	0	0	7
Trunk								
Growth	4	0	0	1	2	0	0	7
Irritation	3	4	2	0	0	0	1	10
Incidental	3	7	8	6	8	9	5	46
Other	0	1	0	0	0	0	0	1
Unknown	0	0	1	0	0	0	2	3
Extremities, proximal								
Growth	1	1	1	0	0	0	0	3
Irritation	0	1	0	1	4	0	0	6
Incidental	0	2	5	3	5	5	1	21
Other	1	0	0	0	0	0	0	1
Unknown	0	0	0	0	0	0	0	0
Extremities, distal								
Growth	6	0	0	2	1	2	0	11
Irritation	1	2	1	0	3	0	0	7
Incidental	2	4	3	3	3	2	0	17
Other	0	0	0	0	0	0	0	0
Unknown	0	0	3	1	0	0	0	4
	30	32	32	25	34	29	18	200

TABLE XVIII

*Clinical Features as Related to Type of Nevus (Total for All Ages)*

Reason removed		Other features		Type of nevus				
				A	B	C	D	E
Growth	39	None	36	2	11	6	4	13
		Irritation	2	0	0	0	1	1
		Other	1	0	0	0	0	1
Irritation	29	None	22	2	1	4	7	8
		Growth	7	1	1	1	2	2
Incidental	112	None	86	2	6	12	24	42
		Growth	11	1	1	0	4	5
		Growth and irritation	2	0	0	0	2	0
		Irritation	2	0	0	0	0	2
		Unknown	11	0	0	0	3	8
Other	6	None	3	1	0	0	0	2
		Growth	2	0	0	0	0	2
		Unknown	1	0	0	0	1	0
Unknown	14	Unknown	13	1	0	1	5	6
		Growth	1	0	0	0	1	0
Totals	200		200	10	20	24	54	92

(D and E) but less often. However, types A, B, and C are characteristic of the first decade in which the common feature is growth. Further analysis of the symptoms as they relate to each age period demonstrates that in any one decade the nevi, no matter what the complaint, are very much alike. The charting of this analysis is lengthy and is not included in this paper.

Nevi of the E type are comparatively acellular and it is reasonable to assume that growth relates to fibrillar proliferation.

*Nevi Which Deviate Histologically from the Trend.* Certain deviations from the trend were mentioned earlier in this analysis and they are readily seen in Table XIV. These deviations are of two types: (I) those occurring in children but resembling nevi of adults (types D and E), and (II) those occurring in adults but resembling those usual in childhood (types A, B, and C). These are analyzed in Table XIX.

No special conclusion can be drawn concerning the first group. Either certain nevi "mature" more rapidly than usual and proliferation at the dermo-epidermal junction ceases early or the growth of nevus cells is entirely from deep sites. Serial sections would probably be enlightening in these cases, but these were not made.

The second group is of more clinical interest. The first 5 cases are in the second decade and are not striking deviations from the trend. From them, however, it is evident that nevi of types A and B may have a duration of at least a decade. All of these nevi date from birth. It is to be noted that nevi of the feet or hands are represented in 3 of these 5 cases. There are 2 cases of type A nevi in adult life. One, from the foot, was stated to be of 1 year's duration. No history was available in the other, which was from the hand. There were 3 type B nevi in adults, of which one was stated to be present since birth, one for 15 years, and one had been noted only a few days. All were present on the feet. The type C group in adults is more variable in location. Four were from toes, 1 from axilla, 1 from abdomen, 1 from elbow, 1 from cheek and 1 from back. All are said to have been present since birth or childhood, or for a long period of time.

In an analysis of age groups beyond 20 years, it is found that the frequency of active growth in nevi which deviate from the trend is greater than the average for all other nevi of adults. In the group which deviates from the trend, 5 of 12 cases in which information was available, or 42 per cent, listed this feature. This is to be compared with 32 of 104, or 31 per cent, in other nevi of adults. These differences are not statistically significant.

The series is too small for conclusions and study of a larger group is

TABLE XIX  
*Nevi Which Histologically Deviate from Trend*

Case no.	Type of nevus	Age	Location	Reason for removal	Group	Stated duration	Recent growth	Follow-up
<i>Group I</i>								
3	D	3	Forehead	Growth		Since birth	Yes	10 yrs.
6	E	9	Neck	Irritation		Since birth	No	1 yr.
17	E	9	Back	Growth		Since birth	Yes	1 yr.
36	E	16	Face	Cosmetic		As long as can remember	No	2 yrs.
44	E	17	Back	Incidental		Unknown	Unknown	Unknown
45	E	17	Back	Irritation		As long as can remember	No	13 yrs.
<i>Group II</i>								
42	B	17	Pubic region	Incidental		Since birth	No	11 yrs.
46	B	10	Abdomen	Incidental		Since birth	No	12 yrs.
59	B	13	3rd toe	Incidental		Since birth	No	19 yrs.
61	A	12	Foot	Incidental		Since birth	No	1 yr.
62	A	12	Palm	Exposed to trauma		Since birth	Yes	6 mos.
67	A	25	Hand	Unknown		Unknown	Unknown	Unknown
92	B	29	5th toe	Irritation		15 yrs.	No	5 yrs.
72	C	24	Axilla	Irritation		Since birth	No	5 yrs.
78	C	27	Abdomen	Incidental		Since birth	No	8 yrs.
93	C	27	Elbow	Unknown		Since birth	No	8 yrs.
115	A	37	Sole	Incidental		1 yr.	Yes*	1 yr.
116	C	37	Toe	Incidental		Since childhood	No	1 yr.
117	C	30	Toe	Growth		Since birth	Yes	2 yrs.
118	B	30	Toe	Incidental		Since birth	No	2 yrs.
119	B	36	Sole	Recent appearance		Few days	Yes	1 yr.
147	C	42	Between 2nd and 3rd toes	Growth		Since birth	Yes	8 yrs.
150	C	41	Toe	Irritation		10 yrs.	Yes	6 yrs.
154	C	50	Cheek	Incidental		Since birth	No	8 yrs.
169	C	59	Back	Incidental		More than 20 yrs.	No	17 yrs.

\* Classed as recent growth because of recent appearance.

indicated. If the patients' histories are correct there is no clear-cut rule, for some of these nevi were stated to be of short duration and others were stated to have been present since birth or childhood, or for an indefinitely long period. However, of the 4 nevi of adults of types A and B in which there is clinical information, there is only one which is stated to have been present since birth. Of the C type, 6 of 9 were said to have been present from birth. This is not appreciably lower than the average of the entire series.

*Nevi of Hands and Feet.* Nevi which deviate from the trend are commonly of the hands and feet, and a separate analysis of these is indicated. The peculiar tendencies of nevi of the distal parts of the extremities to be of the flat ("narrow stratified") type with junctional proliferation in adult life have been noted earlier in this study. Nevertheless, it was shown that there are similarities in the trend to nevi in general. In Table XX the analysis is confined to nevi of the hands and feet, omitting legs and forearms.

In patients beyond the age of 20 years there is a higher incidence of history of growth (including recent appearance) than for all other nevi in adults. For those with nevi of the hands and feet there were 7 of 17, or 41 per cent, who gave this history, while for all other nevi of adults for which there was the necessary information there were 30 of 99, or 30 per cent. In such a small series the difference is not significant.

The incidence, in adults, of short duration is a little higher in this group of nevi than with nevi of other locations. In 4 of 17, or 23.5 per cent, of these cases in which there is clinical information, the duration was significantly shorter than the age of the patient. This is to be compared with 13 of 99 cases, or 13 per cent, in all other nevi of adults in which there is clinical information. The series is small and the difference is not significant.

The above data concerning growth and duration must be weighted by the consideration that there is a special selection for surgical removal in these cases. Nevi of the feet and hands were less frequently removed incidentally and more frequently removed because the patient was aware of growth. In contrast, in cases of nevi of the thorax which were often removed incidentally during an abdominal operation, the patient may not have been aware of the nevus and its growth characteristics.

The eventual trend of nevi of hands and feet in this series is shown in Table XXI which summarizes the appearance of nevi of hands and feet of all ages. It is seen that among nevi located on hands or feet there is a trend toward diminution of the incidence of types A, B, and C between the ages of 40 and 50, and increase of incidence of types D and E. Thus,

TABLE XX  
Analysis of Nevi of Hands and Fingers, Feet and Toes

Case no.	Type of nevus	Age	Location	Reason for removal	Stated duration	Recent growth	Follow-up
<i>Age 0-9 years</i>							
27	B	6	Under toe	Growth	Since birth	Yes	7 yrs.
30	B	2	Hand	Irritation	Since birth	Yes	6 mos.
<i>Age 10-19 years</i>							
57	D	14	Dorsum hand	Incidental	Since birth	No	7 yrs.
58	C	12	Dorsum foot	Bleeding and irritation	Since birth	No	15 yrs.
59	B	13	3rd toe	Incidental	Since birth	No	19 yrs.
60	D	12	Sole	Incidental	Since birth	No	1 yr.
61	A	12	Foot	Incidental	Since birth	No	1 yr.
62	A	12	Palm	Exposed to trauma	Since birth	Yes	6 mos.
<i>Age 20-29 years</i>							
89	E	21	Thumb	Prophylactic and cosmetic	Since childhood	No	3 yrs.
90	D	25	Toe	Unknown	Unknown	Unknown	Unknown
91	A	25	Hand	Unknown	Unknown	Unknown	Unknown
92	B	29	5th toe	Irritation	15 yrs.	No	5 yrs.
94	D	29	Under 2nd toe	Incidental	Since birth	No	1 yr.
<i>Age 30-39 years</i>							
115	A	37	Sole	Incidental	1 yr.	Yes*	1 yr.
116	C	37	Toe	Incidental	Since childhood	No	1 yr.
117	C	30	Toe	Growth	Since birth	Yes	2 yrs.
118	B	30	Toe	Incidental	Since birth	No	2 yrs.
119	B	36	Sole	Recent appearance	Few days	Yes	1 yr.
<i>Age 40-49 years</i>							
147	C	42	Between 2nd and 3rd toes	Growth	Since birth	Yes	8 yrs.
148	E	48	Toe	Incidental	20 yrs.	No	4 yrs.
149	D	41	Toe	Irritation	10 yrs.	Yes	6 yrs.
150	C	41	Sole	Irritation	10 yrs.	Yes	6 yrs.
151	D	48	Sole	Irritation	A number of yrs.	No	5 yrs.
152	D	40	Toe	Incidental	As long as can remember	No	1 yr.
153	E	44	Hand	Incidental	10 or more yrs.	No	10 yrs.
<i>Age 50-59 years</i>							
179	E	51	Between toes	Growth	Since birth	Yes	13 yrs.
181	D	53	Under great toe	Incidental	Since birth	No	10 yrs.

\* Classed as recent growth because of recent appearance.

in this series there is an eventual trend for nevi of hands and feet to lose junctional proliferation and for the cells to differentiate to fibrillar forms, although usually at an older age than is true of nevi of other sites. Study of a larger series of nevi of hands and feet is indicated for these results are not conclusive. It is disturbing that there is a scarcity

TABLE XXI

*Summary of Histologic Appearance of Nevus of the Hands and Feet at Different Ages*

Age group (years)	Type of nevus				
	A	B	C	D	E
0-4	0	1	0	0	0
5-9	0	1	0	0	0
10-14	2	1	1	2	0
15-19	No cases				
20-29	1	1	0	2	1
30-39	1	2	2	0	0
40-49	0	0	2	3	2
50-59	0	0	0	1	1
60-	No cases				

of benign nevi from the hands and feet of patients older than 50 years (we obtained only 2 cases), yet melanoblastomas are common in these sites. Undoubtedly the number is modified by the reasons for removal of a nevus. Most nevi in this series were removed incidentally and incidental removal of a nevus of the hand or foot during a major surgical operation will occur but rarely. Further, if a patient has tolerated such a nevus for 40 or 50 years he probably will not have it removed for cosmetic reasons.

*Significance of Junctional Proliferation in Nevus of Adults.* As pointed out earlier, the data thus far presented comprise only the first step in the analysis of this problem. Several points are evident at this time, however. The presence of junctional proliferation of moderate to marked degree in adult life, especially in young adult life, is to be expected in a minority of cases and of a slight degree in a greater number. There is no sharp distinction of such nevi from all others. There is no age at which junctional proliferation abruptly stops. Therefore, junctional proliferation does not in itself mean a "precancerous" state. A marked deviation from the trend, however, in the degree of this proliferation or in the presence of other variations, as noted in the next paragraph, raises the question of melanoblastoma.

Nevi, of adults, which consist of junctional and intra-epidermal cells almost entirely (type A), are disturbing to the pathologist and although the data submitted do not fully substantiate the conclusion in all instances, it is still suspected that these histologic characteristics indicate recent origin and growth. The other alternative is that a nevus resembling

that of early childhood can persist without further differentiation for as long as 30 to 40 years. Finding nevi (benign) of this type after age 40 is decidedly infrequent. Although the pathologist is guarded in excluding melanoblastoma in these cases, it is thought that until more conclusive evidence is obtained, such a diagnosis must rest on additional observations, including (a) excessively large, irregularly scattered or otherwise bizarre masses or nests of cells at the dermo-epidermal junction, (b) deep penetration of large cells without differentiation to small nevus cells and fibrillar forms, (c) more mitotic figures than are usual, (d) atypical and pleomorphic cells, (e) invasion, and (f) inflammation, other than folliculitis, which is not accounted for by trauma.

### SUMMARY

There are progressive changes in the histologic appearance of nevi throughout life. The features which change most noticeably are size, number of nevus cells, proliferation of cells in the epidermis and at the dermo-epidermal junction, number of mitotic figures, and presence of fibrils and nerve-like elements. In most instances, an approximate correlation of the histologic appearance with the age of the patient can be made by an evaluation of these features.

Judged from the transitions noted in the different age groups, nevus cells appear to differentiate slowly from foci of clear cells found in the epidermis and follicles and along sweat glands. Further differentiation, principally in adult life, leads to the formation of fusiform cells with fibrils which in many instances resemble neurofibrils and tactile corpuscles. Such structures are therefore to be considered the final stage of differentiation of nevus cells, not the source of nevus cells. That the foci in the epidermis, follicles, and sweat glands are the only source of nevus cells is favored but not proved.

There are exceptions to the general trend. Nevi of children may resemble those of early adult life. Nevi of adults, usually in the third and fourth decades, may resemble nevi of childhood. The latter exception is notable in regard to nevi of the hands and feet. However, in this series the trend of nevi in middle age in these sites is similar to the trend of nevi in general. Final conclusions must be based on a larger series.

Junctional proliferation in nevi of adults does not in itself indicate melanoblastoma. There are an expected incidence and degree of such proliferation at various ages. Certain additional criteria must be fulfilled before a histologic diagnosis of melanoblastoma is warranted.

In adults the occurrence of nevi which morphologically resemble those of childhood leads to the suspicion that they are incipient and



growing, but clinical evidence obtained in this analysis of their duration and rate of growth is variable and conflicting, permitting no final conclusion. Evaluation must await further study.

Nevi vary in appearance according to location. A notable modifying factor is the number of hair follicles and sweat glands in the various cutaneous sites. While most nevi have nevus cells in a superficial stratum beneath the epidermis, deeply placed nevus cells tend to have a perifollicular and periglandular distribution. Diffusely scattered, deeply placed nevus cells also occur.

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[ *Illustrations follow* ]

## DESCRIPTION OF PLATES

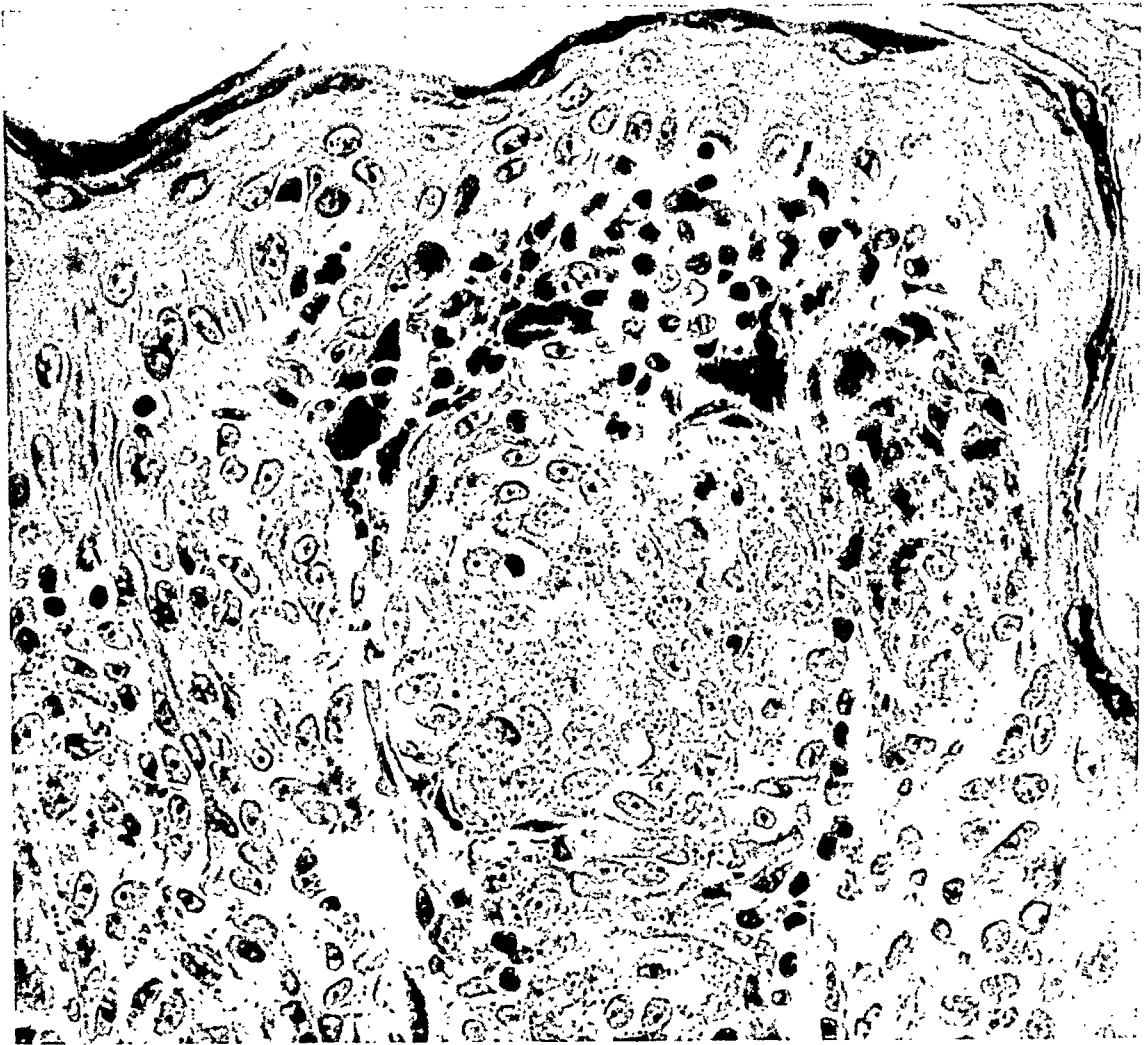
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### PLATE 177

FIG. 1. Nevus of abdomen of a male, 6 years of age. There is proliferation of large pigmented cells at and beneath the dermo-epidermal junction. One mitotic figure is shown.  $\times 445$ .

FIG. 2. Nevus of thorax of a female, 42 years old. Fibrillar structures resemble tactile corpuscles.  $\times 312$ .

1



2

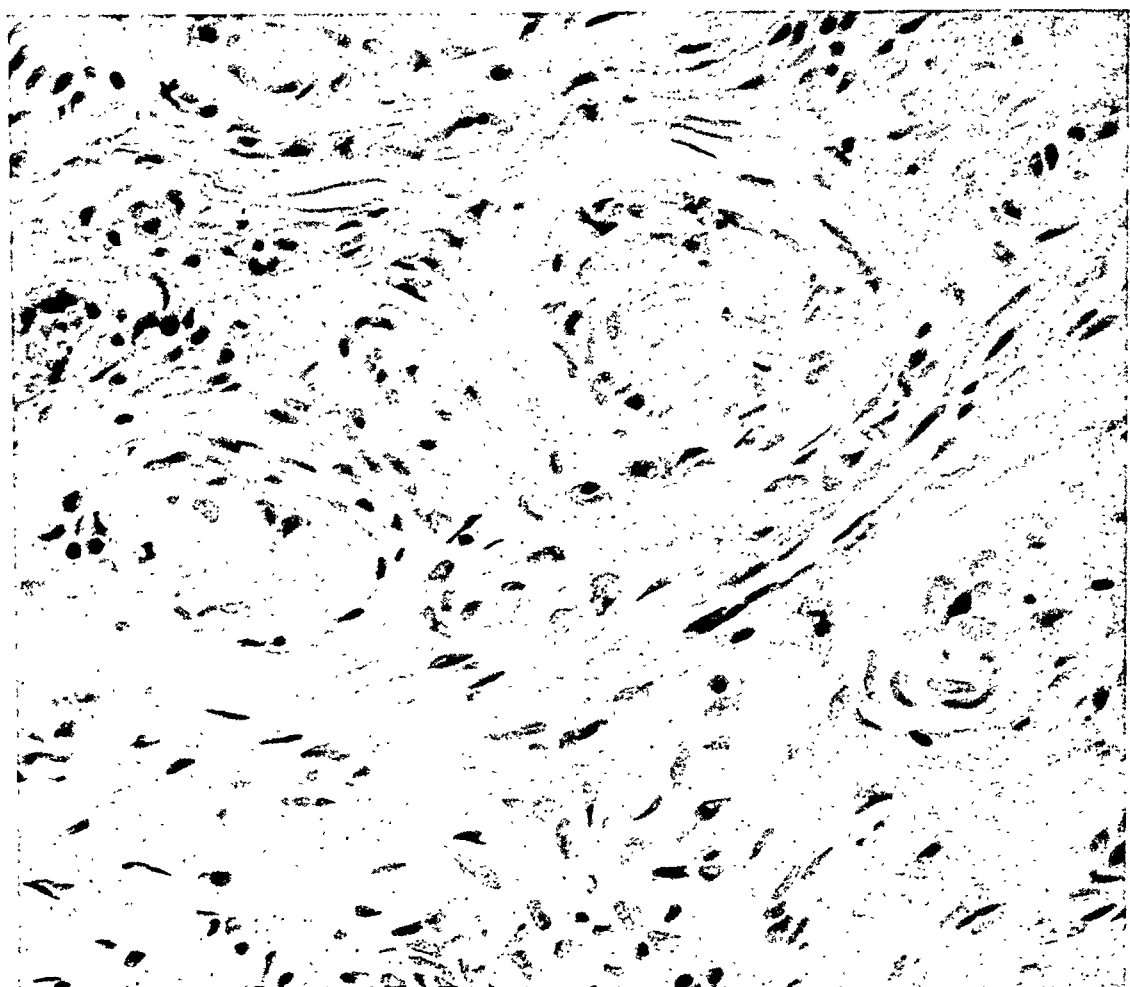


PLATE 178

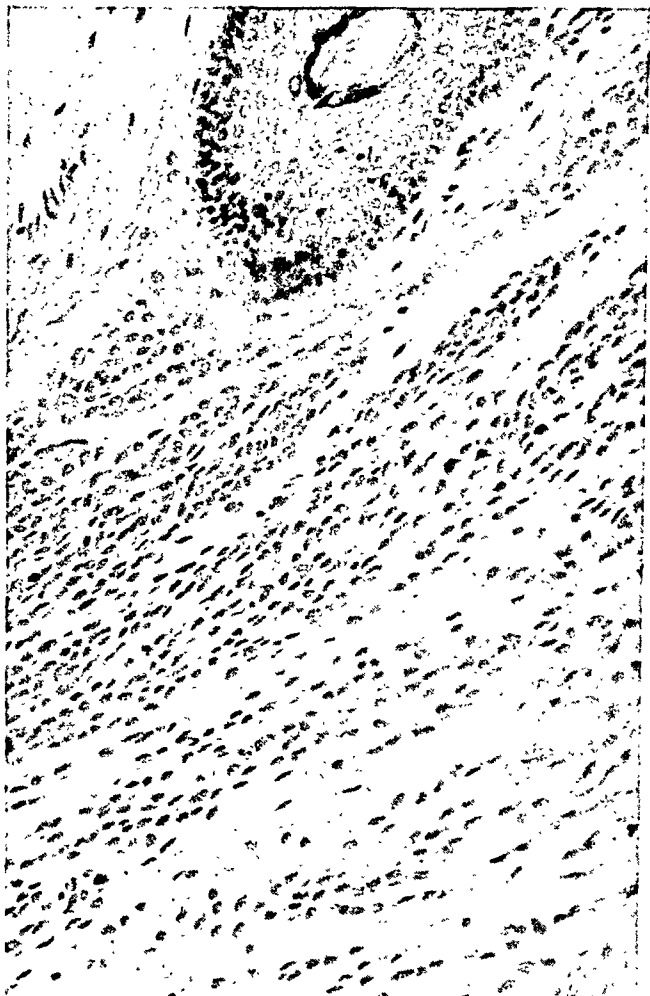
FIG. 3. Nevus of face of a female, aged 12 weeks. Clear cells are present in clumps within the epidermis and along the hair follicles.  $\times 154$ .

FIG. 4. Nevus of a female, 37 years old. Small round nevus cells and fusiform fibrillar nevus cells are present.  $\times 186$ .

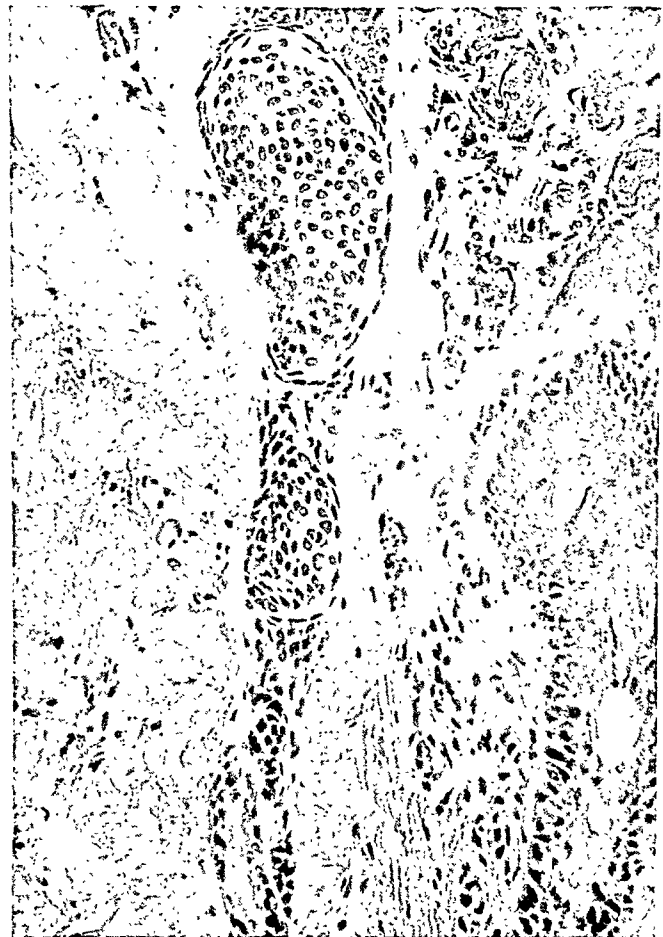
FIG. 5. Nevus of thorax of a male, aged 6 years. Clear cells occur in clumps along a sweat gland.  $\times 169$ .

FIG. 6. Nevus of scalp of a female, 18 years of age, with nevus cells distributed about a sweat gland.

3



5



Lund and Stobbe

Natural History of the Pigmented Nevus

PLATE 179

FIG. 7. Nevus of neck of a female, 19 years old. The deep cells have a perifollicular distribution.  $\times 18$ .

FIG. 8. Nevus of lower eyelid of a male, aged 22 years. Nevus cells are found among striated muscle fibers. There has been no recurrence in 18 years.  $\times 109$ .

7



8

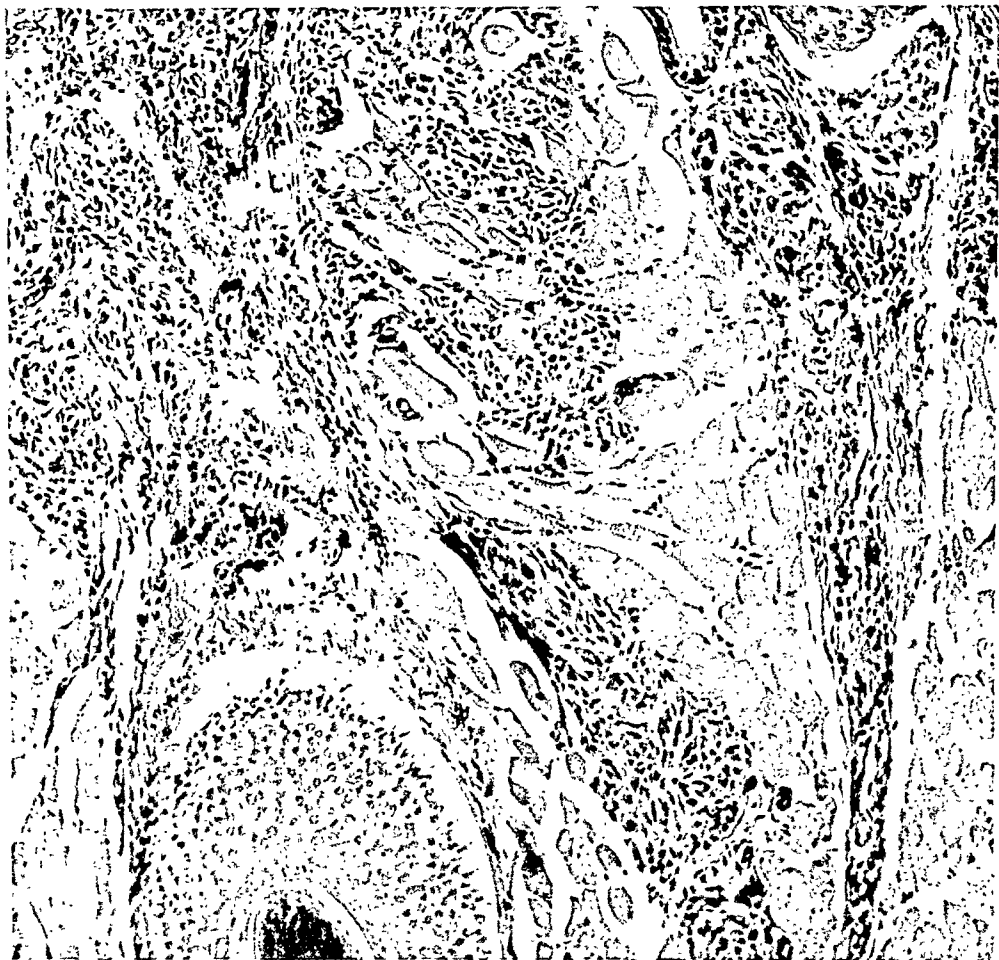




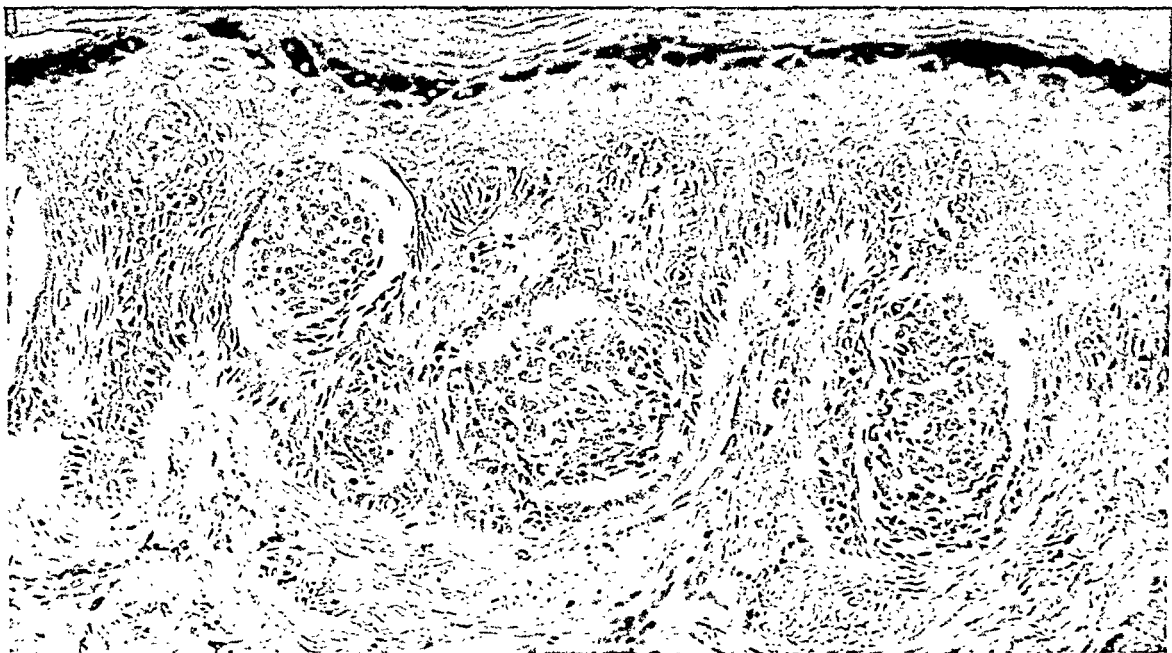
PLATE 180

FIG. 9. Nevus of sole of a female, 37 years of age. Clear cells are present in clumps in epidermis. No nevus cells are found in the corium.  $\times 150$ .

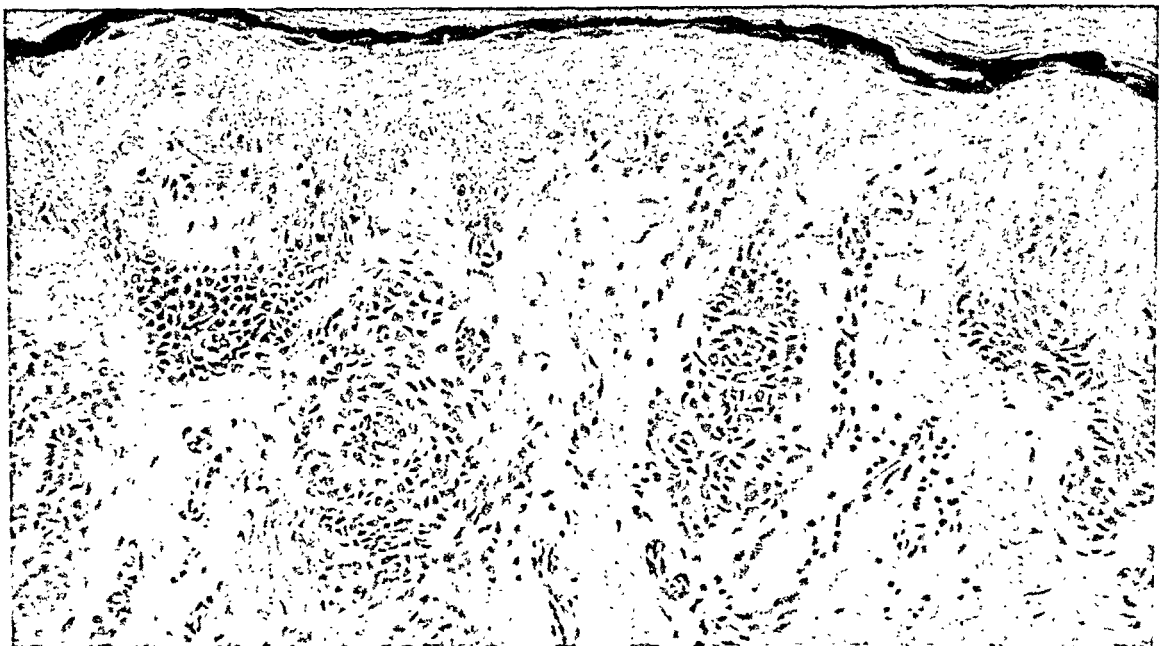
FIG. 10. Nevus of sole of a female, 48 years of age. Nevus cells are present in the corium. There is slight dermo-epidermal proliferation.  $\times 150$ .

FIG. 11. Nevus between toes of a male, aged 41 years. Nevus cells are present in the corium. There is no junctional proliferation.  $\times 79$ .

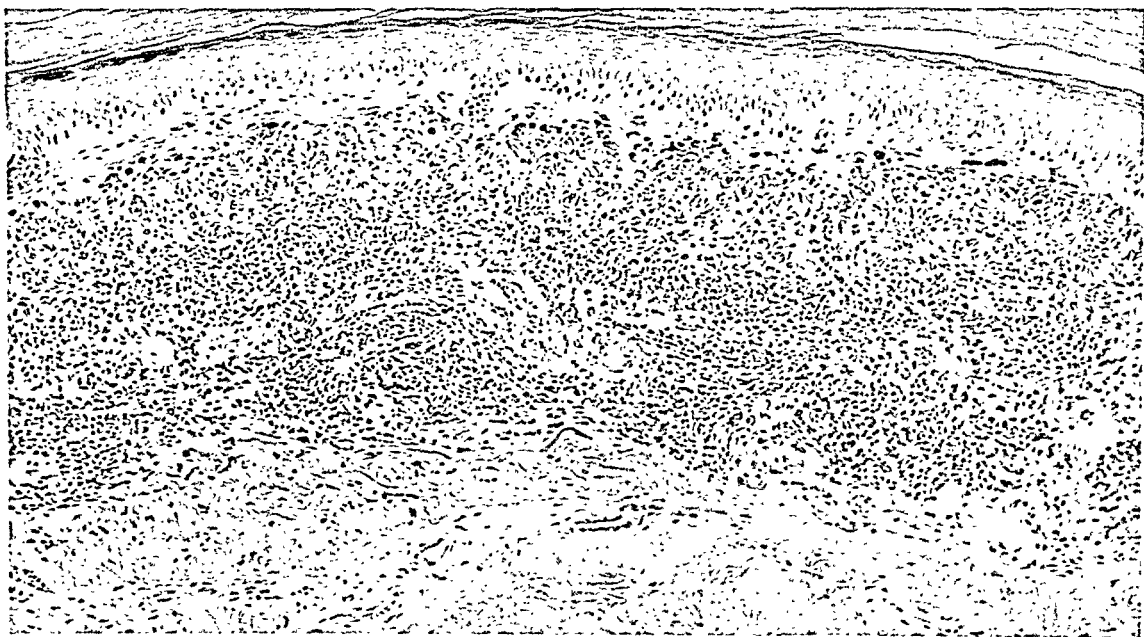
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10



11





## MYOBLASTOMA\*

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Myoblastomas are tumors consisting of large cells with granular cytoplasm which resemble myoblasts, the embryonic precursors of striated muscle fibers. These tumors are commonest in voluntary striated muscle but have been found also in sites where striated muscle is not normally present. The tumors are rare and are mostly benign. Since 1926, when the entity was first recognized, there has been controversy as to their nature, histogenesis, and possible malignancy.

In the present study 10 new cases are reported and reports of 219 other cases from the literature are reviewed.

Abrikossoff,<sup>1</sup> in 1926, has been credited generally with first establishing this tumor entity under the name of "Myoblastenmyome." Several authors<sup>2-7</sup> preceded him with case reports in which the lesions were given various names, but the tumors described are seemingly identical with the entity named and established by Abrikossoff. He was convinced that the large granular cells which made up the bulk of his tumors were identical with the embryonic striated muscle cells, the myoblasts, as depicted by Godlewski<sup>8</sup> in 1902 in his work on the histogenesis of striated muscle.

There have been numerous reports and reviews on this subject since publication of Abrikossoff's paper.<sup>1</sup> Among the more significant contributions, those of the following authors may be mentioned: Klinge,<sup>9</sup> Diss,<sup>10</sup> Meyer,<sup>11, 12</sup> Klemperer,<sup>13</sup> Gander,<sup>14</sup> Cappell and Montgomery,<sup>15</sup> Grayzel and Friedman,<sup>16</sup> Leroux and Delarue,<sup>17</sup> Holle,<sup>18</sup> Ringertz,<sup>19</sup> Horn and Stout,<sup>20</sup> Lauche,<sup>21</sup> Howe and Warren,<sup>22</sup> Crane and Tremblay,<sup>23</sup> Ravich, Stout, and Ravich,<sup>24</sup> Ackerman and Phelps.<sup>25-26</sup> As will be discussed later, the histogenesis and possible malignancy of myoblastomas have been the points of greatest interest to the various observers.

The specimens in this study were obtained from the Section on Pathologic Anatomy and the Section on Surgical Pathology of the Mayo Clinic. All had been preserved in a 10 per cent solution of formalin (3.8 per cent aqueous solution of formaldehyde gas). Sections were cut by the fixed frozen-tissue technic and from paraffin blocks. The frozen sections were stained for fat with sudan III; the paraffin blocks were stained with hematoxylin and eosin, Masson's trichrome, Bodian's stain (silver stain)

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and with a modified Mayer mucicarmine technic.<sup>27</sup> Early in the study some sections were stained with Mallory's phosphotungstic acid hematoxylin and also with Heidenhain's iron hematoxylin, but these yielded no further information and were not continued. Stains for glycogen were not possible because of the fixation with formalin.

## REPORT OF CASES

### *Case 1*

A white man, 62 years of age, was admitted on September 1, 1942, for investigation of a fistula in ano. In the course of routine examination, a nodule was noted on the tip of his tongue; the patient had been unaware of its existence and had no complaints referable to the tongue. Family and past histories were irrelevant. Serologic tests for syphilis gave negative results. The nodule was excised.

*Pathologic Findings.* The gross appearance was not distinctive. The nodule was firm and round and measured about 0.5 cm. in diameter. It cut easily; the cut surface was homogeneous and a dirty white. There was no distinct capsule. On histologic examination, the nodule was seen to consist of large cells with coarsely granular cytoplasm and delicate, often indistinct, cell membranes (Fig. 1a). The nuclei were usually single; they stained lightly with hematoxylin; and each had a visible, though not unduly prominent, nucleolus. In some areas several granular cells seemed to have fused to form a syncytial mass roughly cylindric in outline and containing many nuclei. Irregularly placed between the granular cells were wisps of delicate, acellular connective tissue. The granular cells abutted against the surface epithelium and extended to, and seemed to invade, the underlying muscle. The overlying epithelium was hyperplastic and showed some parakeratosis. In the deeper part, where the granular cells apparently were invading the muscle, some seemingly degenerating muscle fibers were seen; but although the granular cells and altered muscle fibers were frequently intimately related, no actual transition from one to the other could be demonstrated. Nor were longitudinal or cross striations seen in the granular cells. The cytoplasm of the granular cells was acidophilic; in some, round bodies were noted which were 3 to 4  $\mu$  in diameter, homogeneous, and rather more acidophilic than the surrounding cytoplasm. No mitotic figures were seen.

### *Case 2*

A white man, 52 years of age, was admitted on November 7, 1944, complaining of a painless lump on his tongue which had grown slightly since he first noticed it some 4 months previously. His mother had died from the effects of cancer of the mouth. The past history was irrelevant except that he had been an ardent chewer of tobacco. Examination revealed a round, hard, raised, nontender nodule on the right side of his tongue. Serologic tests for syphilis gave negative results. The nodule was excised.

*Pathologic Findings.* The excised tumor formed a firm, homogeneous, gray-white mass, 1.3 cm. in diameter, with no obvious capsule. On histologic examination the surface epithelium was seen to be markedly hyperplastic. Just beneath this epithelium was a layer of collagenous connective tissue, enmeshed in which were large polyhedral cells with granular cytoplasm and delicate cell membranes. As in case 1, areas were seen where these cells had fused to form a syncytium with numerous nuclei. The musculature of the tongue was extensively infiltrated with the granular cells and there were many altered or degenerating muscle bundles. In a single muscle bundle could be seen normal muscle fibers, altered muscle fibers, and the pale granular cells. It was difficult to evaluate the nature of the change in these "altered" muscle fibers; they stained less intensely and had lost or were losing their striations, but their nuclei were often intact and were sometimes centrally located. They may have been undergoing degeneration or possibly were regenerating as a result of minimal trauma. In some instances a single sarcolemma seemed to enclose granular cytoplasm with nuclei and rather frayed-looking striated fibers (Fig. 1*b*). In this section it seemed quite logical to assume that the degenerating or regenerating muscle fibers actually formed the granular cells. No mitotic figures were seen.

### Case 3

A white woman, 36 years of age, was admitted on August 15, 1945, with varied complaints among which was that of a growth on her tongue. This had been noticed about 1 week prior to admission but, apart from creating a fear of cancer, had caused the patient no distress. Family and personal histories were irrelevant. Examination revealed a small, hard, nontender nodule on the right side of the tip of the tongue. The results of serologic tests for syphilis were negative. The nodule was excised.

*Pathologic Findings.* Grossly, the tumor was similar to those in cases 1 and 2. Histologic examination revealed a decidedly hyperplastic epithelium overlying a mass of the large granular cells, many of which were fused to form cylindric syncytia (Fig. 2*a*). The cytoplasm was coarsely granular and contained the amorphous round bodies noted in case 1. In the deeper part of the tumor, the granular cells seemed to infiltrate the muscle layer. No transitional forms were observed, but many of the cylindric granular masses seemed to have an alignment of their granules very suggestive of longitudinal and cross striations. No mitotic figures were noted. The connective tissue stroma was even less prominent than in cases 1 and 2.

### Case 4

A white man, 41 years of age, was admitted on June 28, 1946, complaining of a painless, slowly growing mass in the left side of his tongue which had been present

for about 1 year. On examination, a hard, nontender, circumscribed mass about 0.5 cm. in diameter was noted in the left margin of the tongue. The nodule was excised.

*Pathologic Findings.* The excised nodule was firm and dull white on cut section which measured about 0.7 by 0.5 cm. Histologic examination revealed a picture similar to that described in case 1. There were the same aggregations of large granular cells in intimate relation to the overlying markedly hyperplastic epithelium (Fig. 2*b*) and the same infiltration of the muscle in the deeper zones. In some areas there was close contiguity of granular cells and somewhat altered muscle fibers, but no very convincing transitional forms were noted. The round, amorphous cytoplasmic inclusions were prominent in many of the granular cells. No mitotic figures were seen.

### Case 5

A white man, 43 years of age, was admitted on April 15, 1947, complaining of a lump in his tongue which had been present for about 3 years. This had increased in size gradually but had not ulcerated. Early in March, 1947, his local physician had taken a specimen for biopsy and had told him he had cancer of the tongue which would require surgical treatment. Family and personal histories were irrelevant. The results of serologic tests for syphilis were negative. On examination, a hard mass measuring about 2 by 3 cm. was seen on the left anterior border of the tongue; the surface showed a whitish plaque (Fig. 3*a*). Two other plaques, each about 0.5 cm. in diameter with underlying firmness, were noted on the right side of the tongue. On April 17, 1947, a specimen was excised and diagnosed "grade 2 squamous-cell epithelioma." The next day a dissection of the submental and bilateral submaxillary nodes, including the salivary glands, was performed; no metastases were found in the tissues removed. It had been the intention to do a plastic operation on the tongue later, but the patient died rather suddenly some 16 hours after operation.

*Pathologic Findings.* At autopsy the cause of death was established as edema of the larynx. There were no other pertinent findings except as related to the lesions of the tongue. The larger tumor on the left side of the tongue was gray-white on cut section, with an indefinite margin (Fig. 3*b*). The two smaller plaques seemed to be limited to the surface epithelium, the underlying tissues appearing quite normal. Histologically, the main tumor was seen to consist of sheets of large, ill defined granular cells similar in all respects to those seen in the other lingual myoblastomas (Fig. 3*c*). The overlying epithelium was greatly hyperplastic and in several areas showed early squamous cell carcinoma. The tumor cells were infiltrating the underlying muscle and numerous transitional forms were noted (Fig. 3*d*). The two smaller "tumors" showed merely some epithelial hyperplasia with edema of the underlying tissues.

### Case 6

A mulatto woman, 72 years of age, was admitted on October 16, 1946, with numerous complaints, mostly of senile origin. In the course of routine examination

a hard, nontender, freely movable nodule about 2 cm. in diameter was noted in the right labium majus pudendi. Family and personal histories were irrelevant. At operation the nodule was found to be attached to the overlying skin at one point but did not seem to invade the deeper tissues.

*Pathologic Findings.* The excised nodule measured 3.5 by 2.5 by 2 cm., was of firm consistency, and the cut surface was white. On histologic examination the nodule was seen to consist of the typical large granular cells along with a collagenous connective tissue rather more prominent than that seen in cases 1 through 5 (Fig. 4a). There was no striated muscle in the sections, nor could cross or longitudinal striations be demonstrated in the granular cells. The overlying skin showed a moderate degree of hyperplasia with parakeratosis, and the subepithelial layer was infiltrated with round cells which were mostly lymphocytes.

### Case 7

A white woman, 37 years of age, was admitted on February 2, 1942, with numerous complaints of a neuropsychiatric nature and also of a lump behind her right ear. This nodule had been present for about 2 years but had shown recent rapid enlargement. Family and personal histories were irrelevant. Examination revealed a firm, slightly movable, subcutaneous nodule about 2 cm. in diameter just anterior to the tip of the right mastoid process. At operation the nodule was found to be densely adherent to the underlying tissue.

*Pathologic Findings.* Grossly, the nodule was firm, white on cut section, and measured 2.5 cm. in diameter. Histologically, it consisted of masses of large granular cells separated by a considerable amount of collagenous connective tissue and numerous thin-walled blood vessels (Fig. 4b). There was neither epithelium nor muscle in the sections examined. None of the granular cells showed cross or longitudinal striations, but a few slender spindle cells showed an alignment of cytoplasmic granules suggestive of striations. No mitotic figures were observed.

### Case 8

A white man, 50 years of age, was admitted on February 19, 1945, complaining of the recurrence of a lump on his left arm. Three years previously in another community, a mass the size of a baseball had been removed from his left biceps muscle, and this tumor had been reported as "grade 4 sarcoma"; an axillary node removed at the same time was not involved. Family history and the remainder of the personal history were irrelevant. Examination revealed a healed scar over the belly of the left biceps; beneath the scar was a firm tumor about 2 by 5 cm. which moved with the muscle but which was not attached to the skin. No enlarged nodes could be palpated in the axilla. At operation a tumor was found embedded in the long head of the biceps; it was removed along with some surrounding, apparently healthy, muscle.

*Pathologic Findings.* The excised tumor was firm, fairly well circumscribed, and measured 2.5 by 2 by 2 cm. Histologic examination revealed a pleomorphic pattern dominated by large cells with granular



cytoplasm (Fig. 5a). These cells varied from about 20 to 50  $\mu$  in diameter. Some very finely and some coarsely granulated varieties were noted, the latter being markedly acidophilic. Some of the larger cells contained multiple nuclei and some a single, large, irregular vesicular nucleus. The nucleoli in all cells were very prominent. For the most part these granular cells were more isolated by the connective tissue stroma than those in cases 1 through 7, and they showed no tendency to fuse into syncytial masses. There was considerable edema of the connective tissue stroma and the tumor in general was quite vascular. Mitotic figures, including abnormal forms, were seen (Fig. 5b). The granular cells were mostly round or oval, but some of the smaller ones were spindle-shaped. Well into the center of the tumor a few bundles of striated muscle fibers were seen, but it was not possible to demonstrate any close relationship of these fibers to the granular cells. At the periphery of the tumor, muscle bundles and long, cylindric granular cell masses were intimately intermingled (Fig. 5c). The tumor was considered a highly malignant myoblastoma.

*Course.* On July 25, 1946, the patient returned with another lump near the old scar over his left biceps, and a nodule 1.5 cm. in diameter was excised. Grossly and histologically it was identical with the tumor removed at the Clinic. He returned again on June 10, 1947, with a flaccid paralysis of his right arm which had developed during the previous month. A diagnosis of metastasis to the left frontoparietal region of the brain was made in spite of negative roentgenologic findings. He was given a course of roentgen therapy and improved somewhat. Three months later, on September 12, 1947, he was readmitted with complaints of low back pain and mental confusion. This time roentgenograms of the head showed a lesion which was interpreted as a cerebral metastasis of the myoblastoma. The patient's wife refused to allow further roentgen therapy and he returned home.

### Case 9

A white man, 50 years of age, was admitted on May 15, 1945, with a lump in his right hand. Five months previously he had bruised his hand slightly and afterwards noticed some stiffness of the middle and ring fingers, but roentgenograms revealed no bony changes. A few weeks later a swelling developed in the palm of his hand; this was incised but little or no pus was found. Penicillin therapy was instituted empirically with no apparent result. A biopsy performed elsewhere at this time resulted in a diagnosis of inflammatory tissue with the possibility of myxosarcoma. On examination there was a firm, freely movable, subcutaneous mass in the right palm. The overlying skin showed a bluish red discoloration. Roentgenograms showed slight osteoporosis of the metacarpals. At operation, the tumor was excised in its entirety; it was adherent to the tendon sheaths but otherwise shelled out relatively easily.

*Pathologic Findings.* The tumor was removed in pieces having an aggregate mass of about 5 by 4 by 4 cm.; it was firm and dull white on cut section. Histologically, it consisted of a myxocollagenous tissue matrix enclosing numerous fine spindle and stellate cells, and many

large, round or elongated cells with acidophilic granular cytoplasm (Fig. 6a). Some of these large granular cells had a fenestrated periphery resembling the so-called spider cells described in rhabdomyosarcoma of the heart. Most of these granular cells contained but one large, vesicular nucleus, often irregular in outline, with one or two very prominent nucleoli. No definite cross striations could be demonstrated but some of the more elongated granular cells seemed to have an arrangement of their granules suggestive of longitudinal and even cross striations. A few mitotic figures were seen in the granular cells. All sections examined showed polymorphonuclear leukocytes and lymphocytes. There was no muscle present.

*Course.* The patient returned on March 12, 1946, having noted a recurrence of the lump in his hand about 1 month previously. This had doubled in size during the month. On examination, a mass 1 cm. in diameter was seen in the right palm over the flexor tendon of the index finger; it was not tender and moved with the tendon. There was also an enlargement of the palmar surface of the right wrist which had been present for about 9 months, gradually increasing in size. Because the patient was a veterinarian and because of the inflammatory reaction in the original tumor, bacteriologic studies were done. Guinea-pig inoculation and smears and cultures for fungi all gave negative results. *Corynebacterium xerosis* was cultured from the wound. At operation a tumor similar to that found previously was excised; the mass in the wrist was continuous with the one over the tendon sheath of the index finger.

*Pathologic Findings.* The tumor was identical grossly with the one removed in 1945. It formed an aggregate about 5 cm. in diameter and weighed 29 gm. Histologically, it was similar to the original growth, but the granular cells were more numerous, many of them were larger, and there were more so-called spider cells. Longitudinal striations were noted in many of the elongated granular cells but no more than a suggestion of cross striation could be demonstrated. The stroma was denser and did not appear as myxomatous as that in the tumor removed in 1945, and there were fewer of the slender spindle and stellate cells. The tumor was adjudged a malignant myoblastoma.

#### Case 10

A white girl, 12 years of age, was admitted on July 3, 1929, with a lump on her left thigh. She had noticed an aching pain in her leg about 5 months previously and the lump appeared shortly thereafter; it had not increased in size. Family and personal histories were irrelevant. Examination revealed a mass about 10 cm. in diameter on the posterior aspect of the left thigh just above the knee. It was not attached to the femur and was of firm consistency. At operation the tumor was excised from its bed in the muscles of the thigh; it was quite sharply outlined and was removed with little difficulty.

*Pathologic Findings.* Grossly, the tumor measured 8 by 5 by 6 cm., it was firm, and the cut surface was homogeneous and gray-white. It

consisted of a meshwork of spindle cells, some of which were quite plump, and numerous large spindle or round cells with as many as four nuclei, with prominent nucleoli (Fig. 6b). These large cells had a finely granular cytoplasm and a suggestion of longitudinal striations, especially in the tails of the larger elongated forms; no definite cross striations were seen. The tumor had a fairly well defined capsule and there was little infiltration of the surrounding muscle in the sections examined.

TABLE I

*Acceptable Cases of Myoblastoma as Reported in the Literature Listed by Year of Report, Author's Name, and Author's\* Case Numbers*

1854 Weber <sup>7</sup>	1937 Bang <sup>70</sup>
1881 Heurtaux <sup>2</sup>	1937 Cappell and Montgomery <sup>15</sup> ; 3, 4, 5, 6
1894 Massin <sup>3</sup>	1937 Gray and Gruenfeld <sup>71</sup> ; 1, 2, 3, 4, 5
1897 Pendl <sup>5</sup>	1937 von Szathmary <sup>72</sup>
1922 Moschcowitz <sup>4</sup>	1938 von Albertini <sup>73</sup>
1925 Quirin <sup>6</sup>	1938 von Bahr <sup>74</sup>
1926 Abrikossoff <sup>1</sup> ; 1, 2, 3, 4, 5	1938 Ducuing, Ducuing, and Bassal <sup>75</sup> ; 1, 2, 3, 4
1926 Keynes <sup>40</sup>	1938 Frenckner <sup>76</sup> ; 1, 2, 3, 4, 5
1926 Rütz <sup>41</sup>	1939 Civatte and Ducourtioux <sup>77</sup>
1927 Dewey <sup>42</sup>	1939 Dustin <sup>78</sup>
1927 Diss <sup>10</sup>	1939 Eickhoff <sup>79</sup>
1928 Klinge <sup>9</sup> ; 5 cases	1939 Fräsdorf <sup>80</sup>
1928 Kraneis <sup>43</sup> ; 1, 2	1939 Geschickter and Maseritz <sup>60†</sup>
1928 Roffo <sup>44</sup>	1939 Kramer <sup>81</sup>
1929 Jaulin and Grandclaude <sup>45</sup>	1939 Leroux and Delarue <sup>17</sup> ; 1, 2, 3
1929 Kleine <sup>46</sup> ; 1, 2	1941 Grayzel and Friedman <sup>16</sup>
1929 Volkmann <sup>47</sup>	1941 Holle <sup>18</sup>
1930 Diss <sup>48</sup>	1941 Kratochvil <sup>82</sup>
1931 Abrikossoff <sup>49</sup> ; 1, 2, 3, 4, 5, 6, 7	1941 Lascano-Gonzalez <sup>83</sup> ; 1, 2
1931 Bolanos and Despaigne <sup>50</sup>	1941 Tams and Kowles <sup>84</sup>
1931 Ceelen <sup>51</sup> ; 1, 2, 3, 4	1941 Thoma <sup>85</sup>
1931 Dawydow <sup>52</sup>	1942 Bernier, Mann, and Ash <sup>86,87</sup> ; 1, 2
1931 Derman and Golbert <sup>53</sup> ; 1, 2, 3	1942 Iglaue <sup>88</sup>
1932 Jorge, Nudelman, and Gringauz <sup>54</sup> ; 1, 2, 3	1942 Monteiro <sup>89</sup>
1932 Meyer <sup>11</sup> ; 1, 2, 3	1942 Ringertz <sup>19</sup> ; 1, 2, 3, 4, 5, 6, 7
1932 Schirmer <sup>55</sup>	1942 Tuta and Schmidt <sup>90</sup> ; 1, 2, 3, 4
1933 Glasunow <sup>56</sup>	1943 Altmann <sup>91</sup> ; 1, 2, 3
1933 Roffo <sup>57</sup>	1943 Ebert and Slepian <sup>92</sup>
1934 Geschelin <sup>58</sup>	1943 Horn and Stout <sup>20</sup> ; 27 cases
1934 Geschickter <sup>59†</sup> ; 14 cases	1944 Howe and Warren <sup>22</sup> ; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10
1934 Kleinfeld <sup>61</sup>	1944 Hartz <sup>93</sup>
1934 Klemperer <sup>13</sup> ; 1, 2, 3, 4, 5, 6	1944 Lauche <sup>21</sup> ; 1, 2, 3, 4, 5, 6, 7
1935 Gander <sup>14</sup>	1945 Crane and Tremblay <sup>23</sup> ; 1, 2, 3, 4, 5
1935 Kernan and Cracovaner <sup>62</sup>	1945 Orr <sup>94</sup>
1935 Martinez <sup>63</sup>	1945 Ravich, Stout, and Ravich <sup>24</sup>
1935 Morpurgo <sup>64</sup> ; 1, 2, 3	1946 Ackerman and Phelps <sup>25-26</sup>
1935 Seiffert <sup>65</sup>	1946 Haagensen and Stout <sup>95</sup> ; 1, 2, 3, 4, 5
1935 Sjögren <sup>66</sup>	1946 Powell <sup>96</sup> ; 1, 2, 3, 4
1936 Bobbio <sup>67</sup>	1947 Khanolkar <sup>34</sup> ; 1, 2, 3, 4, 6, 7, 8, 9, 10
1936 Lattes <sup>68</sup>	1947 Simon <sup>97</sup> ; 1, 2, 3, 4, 5, 6
1936 Parreira and Nunes de Almeida <sup>69</sup> ; 1, 2	

\* The following reports were studied and rejected: von Meyenburg,<sup>28</sup> Lino,<sup>29</sup> Loi,<sup>30</sup> Imperatori,<sup>31</sup> LaManna,<sup>32</sup> Muller,<sup>33</sup> Khanolkar<sup>34</sup> case 5, Cioni,<sup>35</sup> Cooper,<sup>36</sup> The following reports were not available to us: Jorge and Brachetto Brian,<sup>37</sup> Lepage,<sup>38</sup> Cheatele and Cutler.<sup>39</sup>

For data as to sex and age of patient, duration of disease and location, size and histologic type of lesion, see the thesis by one of us (G.H.M.)

† Apparently the cases reported by Geschickter<sup>59</sup> in 1934 and Geschickter and Maseritz<sup>60</sup> in 1939 are the same.

*Course.* There was no recurrence of the tumor on January 17, 1930, and on February 18, 1930, the child died of pleurisy and pneumonia after a short illness. No autopsy was performed, but there was no indication of metastasis.

### ANALYSIS OF CASES

Deciding which cases reported in the literature could be accepted as true myoblastomas was a difficult task. In numerous instances these reports were not documented with photomicrographs or adequate descriptions and some, therefore, may have been included or excluded erroneously. This analysis is based on the 219 cases listed in Table I and the 10 new cases listed in Table II.

TABLE II

*Summary of Data on 10 Mayo Clinic Cases of Myoblastoma Collected by Murphy, Dockerty, and Broders in 1947*

Case	Sex	Age	Duration	Location	Size	Histologic type	Remarks
1	M	62		Tongue	0.5 cm.	Uniform	Epithelial hyperplasia
2	M	52	4 mos.	Tongue	1.3 cm.	Uniform	Epithelial hyperplasia; transitions
3	F	36	1+ wk.	Tongue	Small	Uniform	Epithelial hyperplasia
4	M	41	12 mos.	Tongue	0.7 x 0.5 cm.	Uniform	Epithelial hyperplasia
5	M	43	36 mos.	Tongue	2 x 3 cm.	Uniform	Epithelioma overlying; transitions
6	F	72		Labium	3.5 x 2.5 x 2.0 cm.	Uniform	
7	F	37	24 mos.	Skin, mastoid	2.5 cm.	Uniform	
8	M	50	54 mos.	Biceps	2.5 x 2.0 x 2.0 cm.	Pleomorphic	Mitotic figures
9	M	50	4 mos.	Hand	5+ cm.	Pleomorphic	Mitotic figures
10	F	12	5 mos.	Thigh	8 x 5 x 6 cm.	Pleomorphic	

*Incidence.* As noted above, 219 cases of myoblastoma were found in the literature up to October, 1947. Of the 10 cases encountered at the Mayo Clinic, in 9 the diagnosis was made during the years 1942 through 1947, showing that the entity, while still uncommon, should not be classed with the rarest tumors.

*Site.* Data as to site of tumor were available in all of the 229 cases (Table III), although accurate location was often difficult to ascertain.

For example, a tumor listed as occurring in the thigh might actually be of the skin, subcutaneous tissue, or muscle. This is not always the fault of the recording author, as it is frequently difficult to be sure of the actual tissue of origin. The tongue was by far the commonest site; it was the site in 78 cases (34.1 per cent). The skin was next, with 22 cases

TABLE III  
*Distribution of Lesions by Site in 229 Cases of Myoblastoma*

Site	Cases	Per cent
Tongue	78	34.1
Skin	22	9.6
Breast	18	7.9
Subcutis	16	7.0
Maxilla	16	7.0
Muscle, excluding tongue	11	4.8
Vocal cord	8	3.5
Thigh	8	3.5
Lips	6	2.6
Buttocks	5	2.2
Mandible	4	1.7
Mouth, excluding tongue	4	1.7
External auditory canal	3	1.3
Trachea and bronchi	3	1.3
Vulva	3	1.3
Other sites*	24	10.5
Total	229	100.0

\* Pharynx, arytenoid, axilla, hand, bladder, anus: 2 cases each. Occiput, orbit, lacrimal sac, middle ear, esophagus, urethral meatus, spermatic cord, pectoral region, back, loin, abdominal wall, heel: 1 case each.

(9.6 per cent), followed by the breast (7.9 per cent), subcutis, maxilla, and muscle in that order. As has been noted by others, there seems to be some predilection for the upper respiratory and digestive passages, which were the sites in 124 cases (54.1 per cent), including the 78 cases in which the tongue was the site.

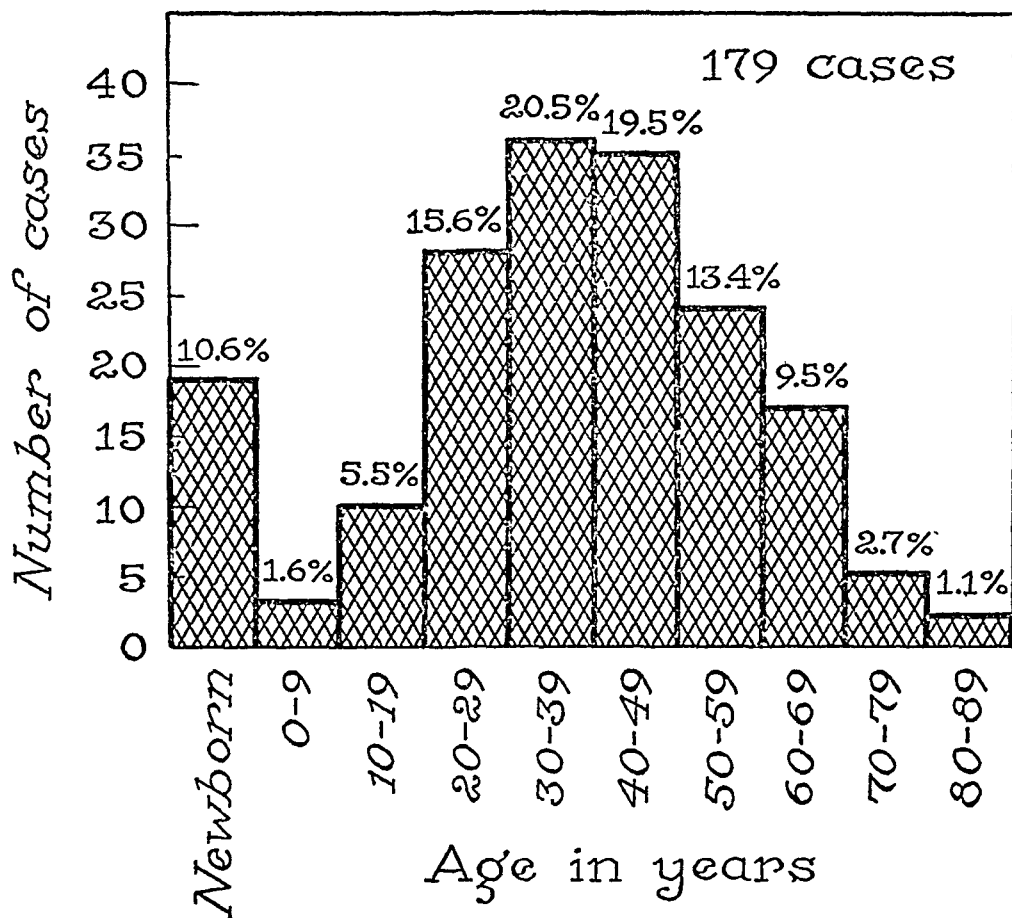
*Age.* Data on age were available in 179 cases (Text-Fig. 1). Apart from 19 "newborn" examples (congenital epulis type), myoblastomas were most numerous during the third, fourth and fifth decades, reaching a peak (20.5 per cent) during the fourth decade.

*Sex.* Sex had no apparent relation to incidence, type, or course of the tumor. In 183 cases in which data were available, 93 of the patients were male and 90 female.

*Morphology.* Grossly, the appearance of myoblastomas is not at all characteristic. In general, they appear as small lumps, often merely as raised and indurated patches as, for example, in the tongue. Occasionally, as when they occur beneath a mucous membrane, they may be pedunculated. They are small tumors, the great majority being between 0.5 and 2 cm. in diameter; the largest recorded was 27.5 cm. in diameter (buttocks<sup>34</sup>). The cut surface is usually homogeneous and

dull white to tan. Sometimes they may have a pseudocapsule owing to compression of the surrounding tissues, but microscopically they have no true capsule.

Histologically, myoblastomas fall into two main groups: uniform and pleomorphic. Abrikossoff<sup>49</sup> originally described four types but, as has



Text-Figure 1. Age distribution of patients who had myoblastoma (179 cases).

been pointed out by Cappell and Montgomery,<sup>15</sup> the distinguishing characteristics of these types are not constant. Thus most tumors described as Abrikossoff's types I, II, and III would seem to be better classified as "uniform" since they consist solely of the granular cells; his type IV, a mixture of spindle cells and a varying number of large granular cells, is covered by the term "pleomorphic." The terms "uniform" and "pleomorphic" were introduced by Howe and Warren<sup>22</sup> in 1944 but they classified their tumors according to Abrikossoff's types. In the 229 cases, 180 of the lesions can be classified as uniform, 13 as pleomorphic, and 36 as undetermined.

The *uniform* myoblastoma consists of sheets or cords of the large granular cells with a small amount of intercellular connective tissue. The granular cells, called myoblasts because of their resemblance to embryonic striated muscle cells, are large, round to oval with a poorly defined

cell membrane, granular and often acidophilic cytoplasm, and a single, central, vesicular nucleus. In some areas, these granular cells seem to fuse to form cylindric syncytial masses with several nuclei. The nucleoli vary considerably in size and are especially prominent in the malignant examples. The granularity of the cytoplasm also varies, being fine and evenly distributed in some, while in others the protoplasm is condensed into small, amorphous balls 3 to 4  $\mu$  in diameter. Staining of these cells for fat and mucus gives negative results but adequate studies on glycogen content have not been done since most or all of the tumors had been fixed in formalin. In myoblastomas found in striated muscle, transitions of adult striated muscle fibers to granular cells have been noted by many observers. In other sites, an arrangement of the granules in a manner suggestive of longitudinal and cross striations is occasionally seen. Frank cross striations are relatively rare and some observers<sup>1,40,70</sup> classify as rhabdomyoma any tumor in which they are present. Mitotic figures have been reported in the granular cells of the uniform type but they are quite unusual.<sup>51,53</sup>

The *pleomorphic* myoblastoma exhibits a very different histologic pattern, having in common only the granular cells, and even these are mostly quite unlike those found in the uniform type. There is a spindle cell stroma with scattered giant cells which are round, oval, strap or tear-drop in shape, with a relatively large amount of granular cytoplasm and one or more central nuclei. These cells have a more distinct cell membrane and are seldom in close contact, being separated by spindle cells. The latter are often plump, have single nuclei, and sometimes show fine but distinct cross striations. Mitotic figures may be seen in both the large granular cells and the spindle cells. This pleomorphic type may grade almost imperceptibly into rhabdomyosarcoma and it is this type that has caused the greatest confusion in the literature. Howe and Warren<sup>22</sup> classified as rhabdomyoma or rhabdomyosarcoma any tumors of this type having "an appreciable number" of cells showing cross striations, while Cappell and Montgomery<sup>15</sup> similarly classified as such tumors those showing "any" cross striations.

A special subdivision of uniform myoblastomas has been described. Horn and Stout<sup>20</sup> noted an arrangement of the granular cells in the form of pseudo-alveoli that gave a picture reminiscent of tumors of endocrine origin. They expressed a belief that in Klemperer's<sup>13</sup> case 6 the lesion presented a similar picture, and Hartz<sup>93</sup> reported another example in 1944 (see also Powell's<sup>96</sup> case 3 and Khanolkar's<sup>34</sup> case 1, Table I). Horn and Stout applied the name "organoid" to myoblastomas

with this pattern but were unable to correlate the microscopic appearance with other distinctive findings.

As first noted by Heurtaux,<sup>2</sup> emphasized by Klemperer,<sup>13</sup> and since reported by numerous authors, hyperplasia of the overlying epithelium is a common finding in myoblastomas occurring beneath an epithelial surface. This has been especially common in tumors of the tongue where sometimes it has been so marked as to warrant the diagnosis of squamous cell carcinoma. Six cases of myoblastoma with overlying squamous cell carcinoma are reported in the literature<sup>6,55,64,75,79,88</sup> and in one of the new cases presented herein a similar picture obtained. In this connection it is interesting to note that in only 2 cases<sup>1,21</sup> has ulceration of this overlying epithelium been noted.

*Clinical Course.* Myoblastomas in general are slow-growing tumors and appear clinically as small, painless nodules that have been present for months or years. Frequently they are noticed accidentally by the patient or discovered in the course of routine physical examination. In the average case it is only the fear of cancer that brings the patient to the doctor. Clinically, for the most part, these tumors are benign and do not recur after local excision. Their greater clinical importance lies in the fact that they may be mistaken for some highly malignant neoplasm and unnecessary radical surgical treatment may be undertaken.

One special group of myoblastomas deserves at least passing mention. This is the so-called congenital epulis of the newborn found on the maxilla or mandible. Nineteen examples are reported in the literature and they are in all respects identical with the uniform myoblastoma found in the tongue and elsewhere.

*Differential Diagnosis.* The uniform myoblastoma must be distinguished from xanthoma, nevoxantho-endothelioma, chordoma, oncocytoma, and high-grade squamous cell carcinoma. The pleomorphic type may be confused with desmoid tumor, teratoma, mesodermal mixed tumor of the female genital tract, and giant cell tumor. The pleomorphic type grades into rhabdomyosarcoma from which it is distinguished, by definition, by the presence of an appreciable number of striated muscle cells in the latter.

#### COMMENT

*New Cases.* The 10 new cases reported herein are in all respects similar to those reported in the literature. In 7 the lesions were of the uniform type and in 3 they were pleomorphic. Age and sex were not remarkable. In 5 cases the lesions occurred in the tongue and were of the uniform type; all showed hyperplasia of the overlying epithelium,



and one lesion (case 5) included a low-grade squamous cell carcinoma. Stains for fat and for mucus gave negative results in all cases. Two tumors of the pleomorphic type recurred after apparently adequate excision and one of these showed a probable cerebral metastasis (case 8).

*Histogenesis.* Hypotheses as to the histogenesis of myoblastomas have followed two main lines of thought. The first is that these tumors are true neoplasms composed of the precursor cells of striated muscle arising either by a degeneration-regeneration process from adult muscle fibers or from heterotopic embryonic rests. The second hypothesis is that myoblastomas are not true neoplasms but rather are storage cell tumors composed of histiocytes or similar cells containing protein metabolites and are thus analogous to xanthomas.

The lesions in Abrikossoff's<sup>1</sup> first 5 reported cases, in 1926, were all situated in skeletal muscle and, on the basis of resemblance of the type cells to embryonic myoblasts as depicted by Godlewski<sup>8</sup> and the intimate relationship of these cells and striated muscle fibers in the histologic sections, he called the tumor a "Myoblastenmyome." Most later observers have endorsed this thesis. In the confirmatory evidence presented by these authors, a few points may be noted: (1) The tumor cells have a granular cytoplasm similar to that of early myoblasts. (2) An arrangement of the cytoplasmic granules in rows suggestive of young myofibrils has been noted. (3) Some of the granular cells have shown actual cross and longitudinal striations. (4) Numerous instances have been reported of apparent direct transition of the muscle fibers to granular cells. (5) Limiting membranes of the granular cells frequently are absent, giving the appearance of syncytial formation as in the normal histogenesis of striated muscle. (6) In myoblastomas of the tongue, the granular cells are found immediately beneath the surface epithelium where no muscle is ordinarily found; this supports the theory of neoplasia as against that of simple degeneration. (7) Further presumptive evidence of neoplasia is seen in the local invasiveness, lack of capsule formation, and recurrence of the tumor following apparently adequate excision.

Abrikossoff<sup>1</sup> expressed the belief that these tumor myoblasts arose from adult striated muscle fibers which had undergone a type of partial degeneration, probably initiated by trauma, and then regeneration with neoplasia of the regenerating cells. Diss<sup>10</sup> reached a similar conclusion independently and he and Abrikossoff were supported by later writers. But Klinge,<sup>9</sup> in 1928, reporting the first case of a myoblastoma in the skin, where no striated muscle is normally found, proposed a theory of origin from heterotopic rests of embryonic striated muscle cells. Abri-

kossoff,<sup>49</sup> reporting 7 more cases in 1931, was forced to accept Klinge's theory of origin for the myoblastomas arising where no striated muscle is normally found but retained his belief in a degeneration-regeneration process for those found in the skeletal musculature. Further support for Klinge's theory was given by the recognition of the so-called congenital epulis of the newborn as a typical myoblastoma; it seemed most probable that such a lesion must be of embryonic origin.

Most subsequent observers<sup>98</sup> followed Abrikossoff's explanation given in 1931, accepting both a dysontogenetic and a degeneration-regeneration origin. Montpellier<sup>99</sup> claimed that the adult type of striated muscle fibers often was present in "abnormal" locations—esophagus, prostate, urinary bladder—and that these tumors might arise from such aberrant fibers. Khanolkar<sup>34</sup> also felt it unnecessary to postulate embryonic rests since there were totipotent mesenchymal cells in the vicinity of small blood vessels throughout the body. The latter is not a new concept and in Maximow and Bloom's text<sup>100</sup> it is credited to Marchand.

A few, however, have raised definite objections. Thus Gander,<sup>14</sup> in 1935, expressed a belief that Diss' rhabdomyome granulo-cellulaire and Abrikossoff's Myoblastenmyome were different entities. The former, found only in the tongue, arose from adult striated muscle by an atypical disintegration while the latter might truly arise from myoblasts. In 1937, Gray and Gruenfeld<sup>71</sup> also raised a dissenting cry. After studying Godlewski's work on the histogenesis of muscle they could not agree with Abrikossoff as to the resemblance of the tumor cells to embryonic myoblasts; nor could they, in a study of animal and human embryos, find any cell forms similar to the type cell of the described tumors. They decided that the lingual myoblastomas did arise from striated muscle by a process of partial degeneration and that the cells were not young myoblasts, but that elsewhere the derivation was most uncertain and must be established in the individual case from a demonstrable relationship to parental cells; in this connection they suggested degenerated glandular epithelium as a possible source. Stout<sup>20,101</sup> was inclined to accept an origin from myoblasts but said that tissue cultures by Murray had not proved this conclusively.

In 1939, while reporting 3 new cases of myoblastoma, Leroux and Delarue<sup>17</sup> presented a new histogenetic hypothesis. The whole conception that the granular tumor cells were modified striated muscle elements was, they said, on a purely morphologic basis and hence could not be proved. They could not agree that these cells were myoblasts and they expressed the opinion that the transitional forms were artifact; there was the same apparent continuity with the cells of the overlying epi-

thelium. Nor could they see any proof that these tumors were true neoplasms. Accordingly, they proposed that the granular cells were mesenchymal cells of histiocytic type containing an unknown substance which might be glycogen. The tumor was formed by a local accumulation of these histiocytes as a manifestation of a general metabolic process.

Holle,<sup>18</sup> in 1941, arrived at conclusions similar to the above. He expressed the belief that the so-called myoblastomas were "Speicherzellgeschwülste," storage cell tumors, and that, on the basis of their staining reactions, the granular cells were mesenchymal cells which contained mucus. Ringertz,<sup>19</sup> in 1942, agreed that the so-called myoblastoma was a storage cell tumor but thought that the cells contained the products of protein breakdown rather than mucus. Lauche,<sup>21</sup> in 1944, agreed; staining of the granular cells for fat and mucin, in his experience, gave negative results and he expressed the belief that the granular material was of a protein nature. The tumor was, he said, analogous to a xanthoma.

At present it is not possible to make a definite decision as to the nature of the so-called myoblastomas. The storage tumor hypothesis awaits confirmation or condemnation by precise biochemical studies and cannot be excluded on purely morphologic grounds. Certain observations with reference to an origin from striated muscle cells can, however, be made at this time.

It would seem of primary importance to distinguish between the uniform and pleomorphic types. While these may be histogenetically related, they have different histologic and clinical characteristics, as previously noted. The uniform myoblastoma exhibits cells which are similar in appearance to the forms found in striated muscle that is regenerating after physical or physiologic trauma. Possibly there is a neoplasia of these regenerating muscle cells at their myoblastic or sarcoblastic stage; this is essentially Abrikossoff's contention.

The pleomorphic myoblastoma is unquestionably of myoblastic origin. The granular myoblasts of this lesion are similar to those seen in a teratoma of the ovary which contained both myoblasts and adult striated elements; they differ markedly from the type cells of the uniform myoblastoma. Also notable is the frequent association of granular cells and striated fibers in tumors of this type that have been called myoblastomas. The myoblasts of the pleomorphic type may arise from embryonic rests, but it is unnecessary to postulate rests when it seems so probable that there exists throughout the body primitive mesenchyme capable of differentiating into any type of adult connective tissue.<sup>34,100</sup> There is also the close relationship between the pleomorphic myoblastoma and rhabdomyoma and rhabdomyosarcoma. So close is this relationship that it is

arbitrary to separate them; it would seem more logical to consider the pleomorphic type as a low-grade rhabdomyosarcoma. This view is supported by Stout's<sup>101</sup> contention that striations are not necessary for the diagnosis of rhabdomyosarcoma.

*Malignancy.* The question of malignancy is somewhat confused by the previously noted uncertainty as to classification and diagnostic criteria. All of the cases of so-called malignant lesions reported prior to 1945<sup>15,22,28,30,33,35,59,64,66</sup> (15 cases, according to Howe and Warren<sup>22</sup>) were of pleomorphic type and, from their illustrations and descriptions, some of them should be classed as those of frank rhabdomyosarcoma. But Ravich, Stout, and Ravich<sup>24</sup> in 1945 and Ackerman and Phelps<sup>25-26</sup> in 1946 each reported one case of uniform myoblastoma with widespread metastasis. These cases seem to be instances of true malignant myoblastomas; in one the lesion originated in the urinary bladder and in one, in the gluteal region; both were classified as Abrikossoff's type III lesion. Khanolkar,<sup>34</sup> in 1947, reported 2 cases (cases 1 and 3, Table I) of apparently uniform myoblastoma with metastasis causing death. Most of the pleomorphic and a few of the uniform myoblastomas recurred after surgical removal, but this may well have been a result of inadequate excision due to the infiltrating nature of the tumor rather than an index of the relative malignancy of the cells.

In general, myoblastomas of uniform type are nearly all benign, although malignancy is possible, while those of pleomorphic type often exhibit at least a low-grade malignancy.

*Therapy and Prognosis.* Radiation therapy has been tried with little or no success<sup>20,24,25,34</sup>; surgical removal is the treatment of choice. For the benign uniform myoblastomas, local excision will effect a complete cure; this excision should be relatively wide, however, since the margin of these tumors is difficult to define. Until more is known about the entity, the rare malignant uniform myoblastoma must likewise be treated by simple excision unless the apparent malignancy of the tumor, as evidenced histologically, or its clinical spread demands more radical procedures. The pleomorphic myoblastomas should be treated as malignant neoplasms, with wide local excision being performed, and the patients should be watched closely for evidences of recurrence or metastasis. Definitely malignant pleomorphic myoblastomas behave in all respects like rhabdomyosarcomas, with as varied and uncertain a prognosis.

#### SUMMARY AND CONCLUSIONS

Myoblastoma was established as a tumor entity by Abrikossoff<sup>1</sup> in 1926. There have been numerous reports and reviews in the literature

since that time, from which we have compiled a list of 219 acceptable cases; we have reported 10 new cases, making a total of 229 cases on which our analysis is based.

Since classification on the basis of Abrikossoff's types<sup>49</sup> is unsatisfactory, the following classification is suggested: uniform type, benign or malignant; and pleomorphic type, benign or malignant.

The uniform myoblastoma arises from adult muscle fibers or from pluripotent mesenchyme, or it may possibly be a storage cell tumor analogous to xanthoma. It is rarely malignant and is usually cured by simple excision.

The pleomorphic type arises from pluripotent mesenchyme and is usually of low-grade malignancy; it often recurs after excision. This type is closely related to rhabdomyosarcoma and should probably be so designated.

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## DESCRIPTION OF PLATES

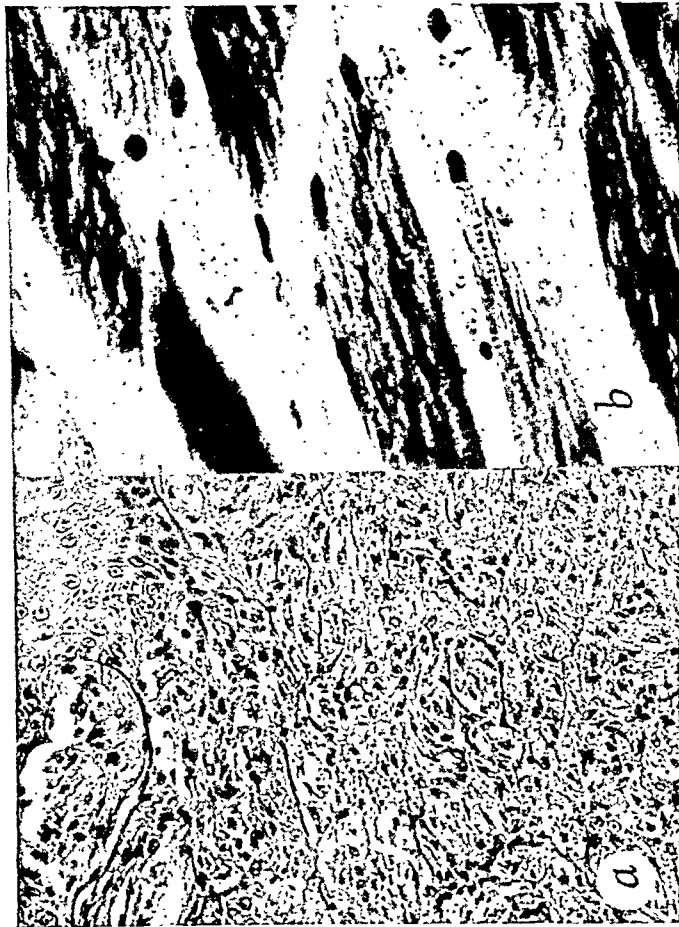
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### PLATE 181

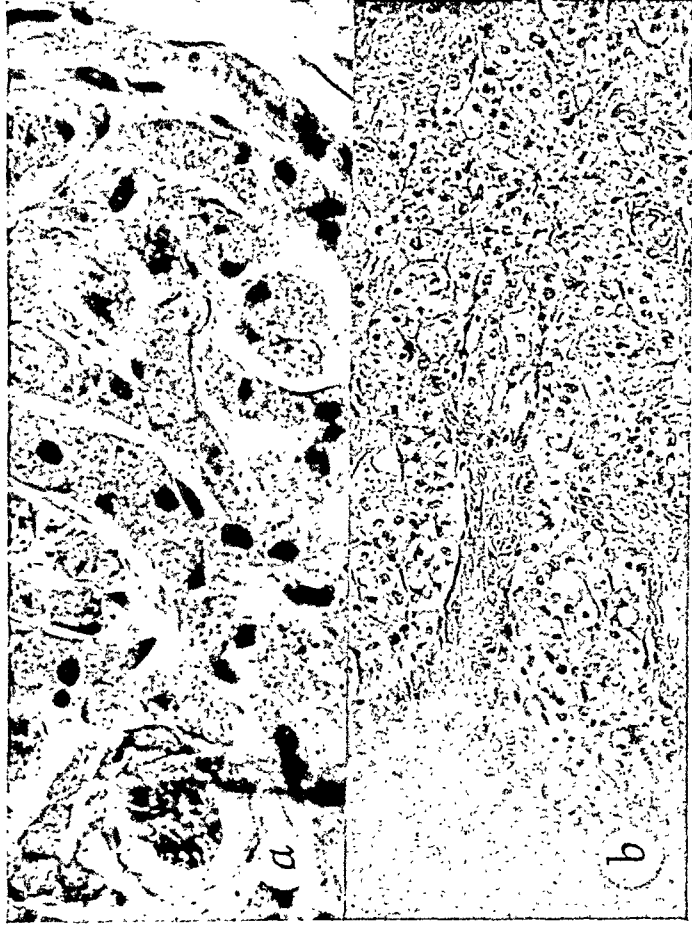
FIG. 1. Uniform myoblastoma. *a* (case 1). A mass of the granular cells is in close relation to the overlying hyperplastic epithelium. Hematoxylin and eosin stain.  $\times 130$ . *b* (case 2). The intimate relationship of granular cells and striated muscle fibers at the periphery of the tumor is apparent. Bodian stain.  $\times 365$ .

FIG. 2. Granular cells of the uniform myoblastoma. *a* (case 3). Fusion to form multinucleated syncytial masses is demonstrated. Bodian stain.  $\times 365$ . *b* (case 4). Hyperplasia of the overlying epithelium. Hematoxylin and eosin stain.  $\times 130$ .

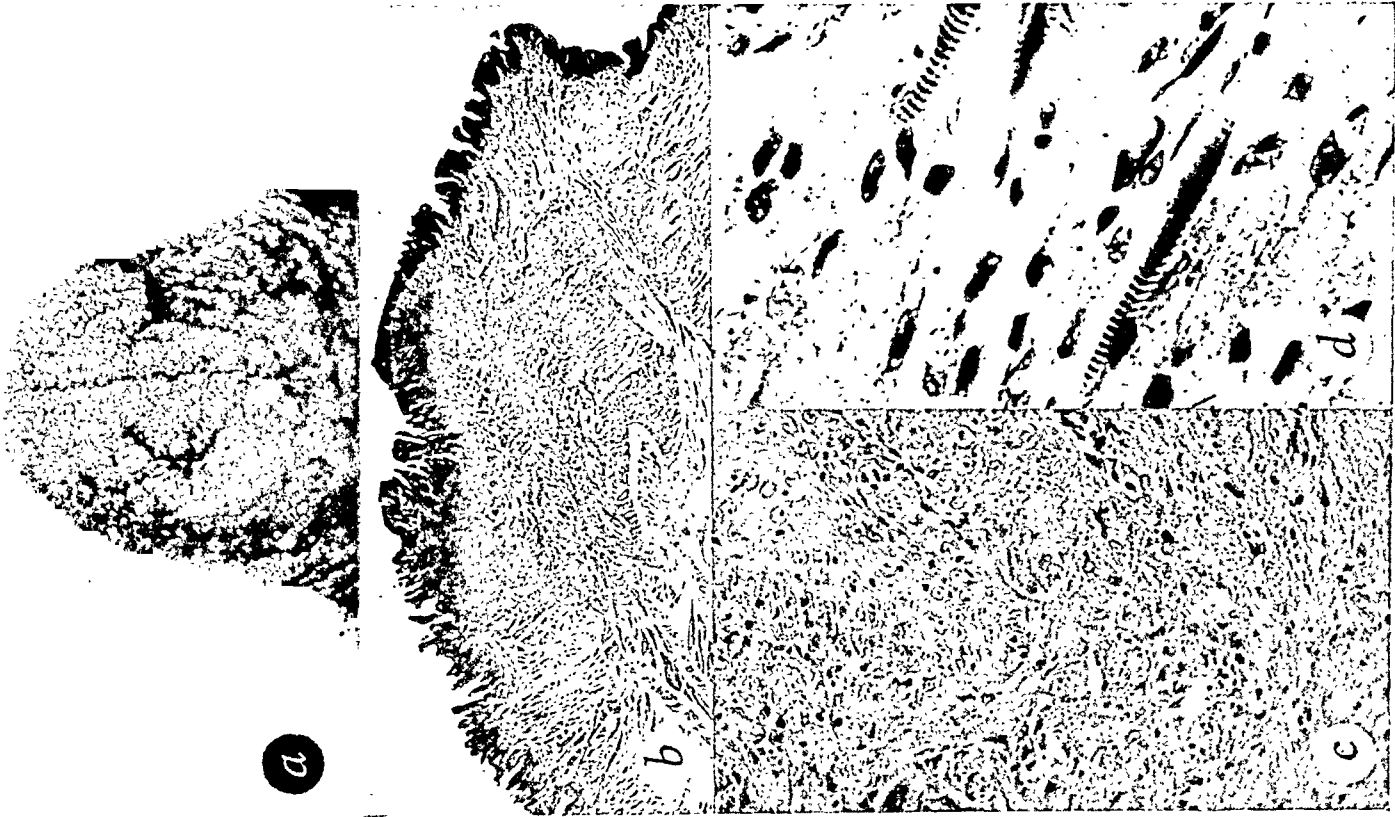
FIG. 3 (case 5). Tongue (autopsy specimen). *a*. A raised, whitish, irregular patch of hyperplastic epithelium overlies a uniform myoblastoma on the left side of the dorsum. *b*. Infiltration and lack of encapsulation are evident. Mallory-Heidenhain stain.  $\times 2\frac{1}{2}$ . *c*. The granular cells lie immediately beneath the squamous cell carcinoma. Hematoxylin and eosin stain.  $\times 130$ . *d*. Transitional forms and intimate relationship of granular cells and striated muscle fibers are shown. Bodian stain.  $\times 390$ .



1



2



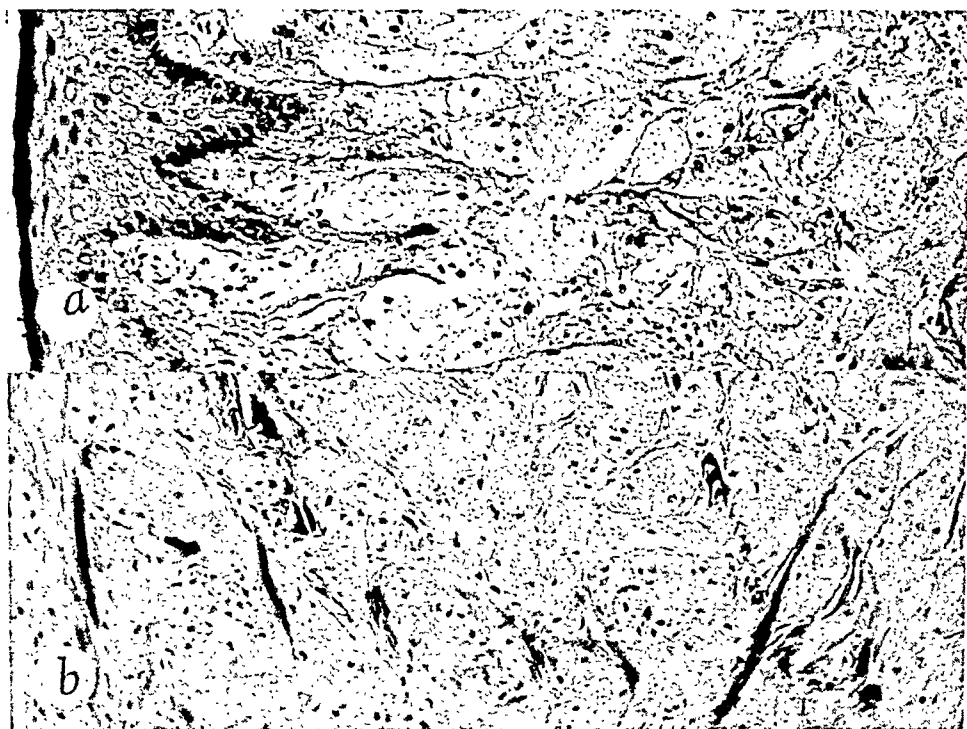
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PLATE 182

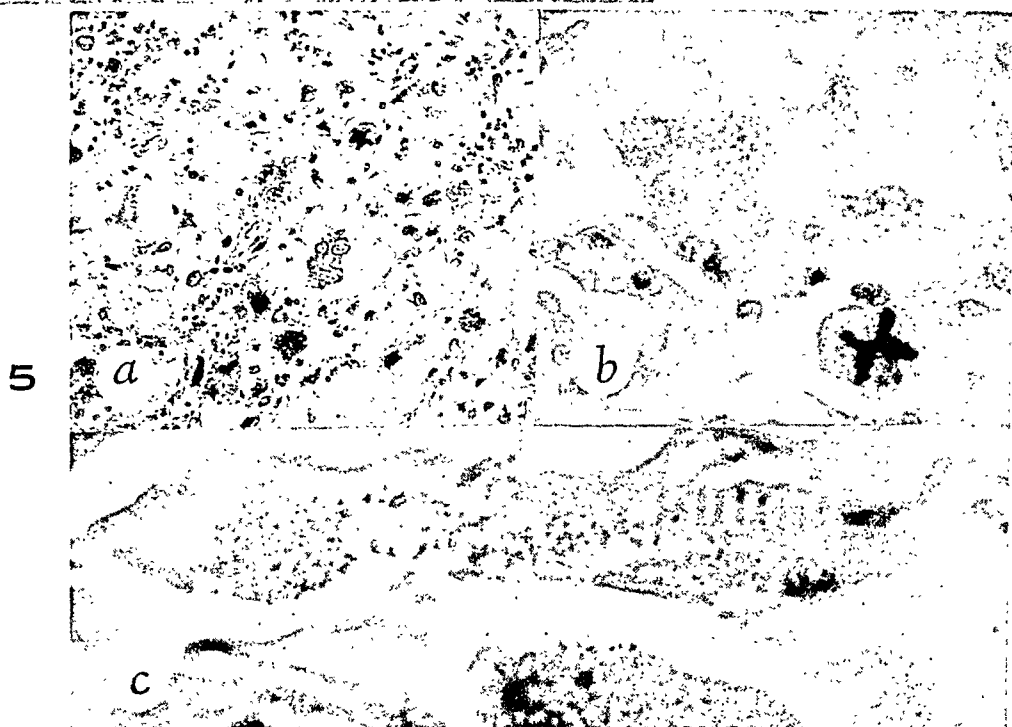
FIG. 4. Uniform myoblastoma. *a* (case 6). Granular cells and overlying epithelium with hyperplasia and parakeratosis are demonstrated. Bodian stain.  $\times 130$ . *b* (case 7). Granular cells are mingled with acellular connective tissue. Hematoxylin and eosin stain.  $\times 130$ .

FIG. 5 (case 8). Pleomorphic myoblastoma. *a*. There are large myoblasts, some with coarsely granular and some with finely granular cytoplasm; many are multinucleated. Hematoxylin and eosin stain.  $\times 130$ . *b*. An atypical mitotic figure is shown. Hematoxylin and eosin stain.  $\times 435$ . *c*. Section from periphery showing a cylindrical granular cell mass with striated fibers in its substance. Bodian stain.  $\times 830$ .

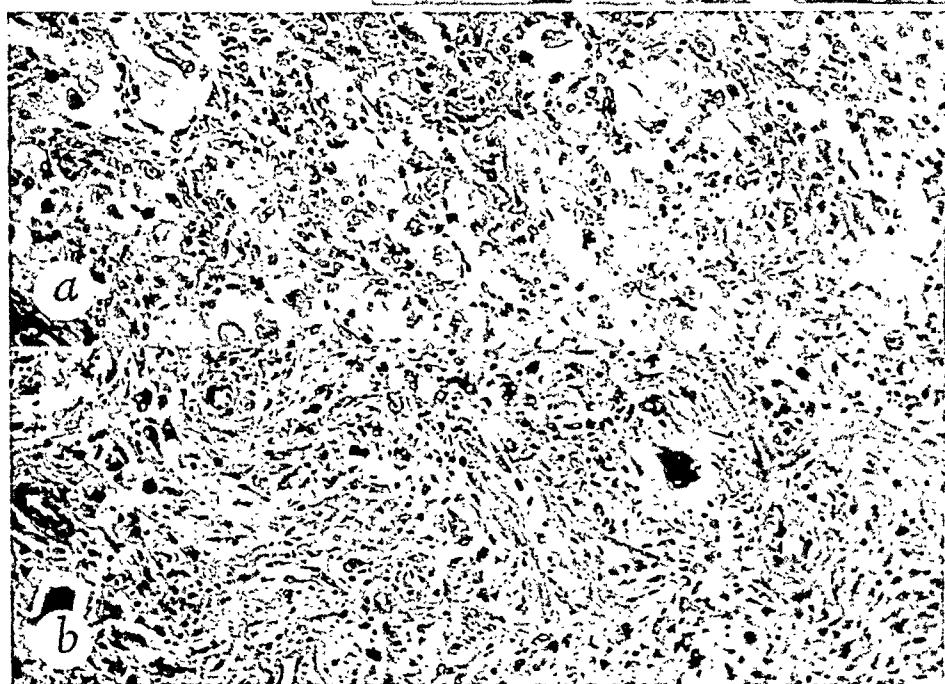
FIG. 6. Pleomorphic myoblastoma. *a* (case 9). Varied types of large cells with granular cytoplasm are shown. Many are round to oval and there are also elongated strap forms. Hematoxylin and eosin stain.  $\times 130$ . *b* (case 10). A stroma of plump spindle cells is shown, with interspersed granular cells and a few multinucleated giant cells. Hematoxylin and eosin stain.  $\times 130$ .



4



5



6



## JUVENILE TUBERCULOUS INFECTION, POSSIBLY OF AVIAN TYPE\*

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Definitely proved progressive tuberculous infections in human beings caused by tubercle bacilli of the avian type are exceedingly rare. The literature contains many alleged cases, but when examined critically few are convincing.<sup>†</sup> That the avian type of tubercle bacillus has seldom been proved responsible for progressive tuberculosis in man is of great interest when one appreciates that avian tuberculosis is an extremely common disease of poultry in large areas of the United States and throughout much of Western Europe. In addition, tuberculosis of swine, due to the avian type, is of common occurrence in areas where the disease occurs in chickens.

The character of the disease in its natural host—the chicken—is such as to provide favorable circumstances for its dissemination to susceptible animals. Its insidious course, its chronicity, the presence within the lesions of prodigious numbers of tubercle bacilli, and the tendency of the infection to produce ulcerative lesions of the intestine from which enormous numbers of the infective bacteria are discharged are important factors accounting for spread of the disease.

While much is known concerning the dissemination of avian tuberculosis to susceptible wild and domesticated animals, much uncertainty exists regarding its occurrence in human beings. Much of the confusion which exists is due to the fact that many alleged cases have been either inadequately described or incompletely or incorrectly studied. In spite of the skepticism that results from a critical examination of the data pertaining to the reported cases, one cannot ignore the fact that in exceptional circumstances avian tubercle bacilli have been the etiologic agent responsible for human infections. Such cases are extremely rare and the validity of their existence can be established only by careful studies using methods that will provide pertinent information regarding the pathogenicity of the acid-fast organisms isolated from the patient.

Typical avian tubercle bacilli are biologically unlike mammalian tubercle bacilli in several respects. The most important of these is their ability to produce, in susceptible hosts, progressive and frequently

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† The literature pertaining to infection of human beings by tubercle bacilli of avian type has been reviewed by Feldman<sup>1,2</sup> and by Rich.<sup>3</sup>

fulminating tuberculosis. Guinea-pigs, markedly susceptible to tubercle bacilli of the human and bovine types, usually resist even massive doses of avian tubercle bacilli given subcutaneously. When mycobacteria from human infections fail to produce progressive destructive tuberculosis in guinea-pigs, one should consider whether the organism is of an avirulent human or bovine type of tubercle bacillus such as may occur in lupus, or whether it is an avian tubercle bacillus, or an atypical strain which has not heretofore been identified. This information can be obtained only by properly conducted tests of pathogenicity.\* When such tests are done properly, the identification of the avian tubercle bacillus may be said to rest on evidence that is valid and convincing.

### REPORT OF CASE

The patient was a female child born on a Minnesota farm in December, 1944. She weighed 9 lbs. (about 4.1 kg.) at birth. The child was breast-fed for more than 15 months, after which she was allowed to choose her own diet. The family used raw milk from their own herd. The animals had reacted negatively to mammalian tuberculin when last examined 5 to 7 years previously.

The child developed normally, having none of the usual childhood diseases. No vaccinations or immunization inoculations had been done. Her one noticeable habit was frequent thumb sucking. She assisted her mother each day in gathering, cleaning, and crating eggs for market, and was particularly fond of playing with the chickens, which were allowed to roam at will about the farm during the summer and fall months.

At the age of 22 months symptoms suggestive of an acute upper respiratory infection occurred on October 4, 1946, and her condition had become alarming by October 10. A physician found the tonsils to be acutely inflamed and the symptoms to be suggestive of pneumonia. Penicillin was administered. The acute reaction subsided but attacks of fever, in which the temperature reached 104° F., and of dyspnea recurred repeatedly during the winter. Certain symptoms referable to the chest persisted and the child was admitted to a hospital in St. Cloud, Minnesota, for observation. Bronchoscopic examination did not reveal bronchial lesions or obstructions, but material swabbed from the bronchoscope was found on examination of smears to contain acid-fast bacillary forms.† A roentgenogram of the chest revealed massive involvement of the upper lobe of the left lung. A diagnosis of tuberculosis was made and the patient was transferred to the Glen Lake Sanatorium on April 29, 1947.

The mother, father, and 4 siblings (aged 10, 7, and 4 years, and 6 months) were examined for tuberculosis. The tuberculin reaction was negative on all except the mother. A subsequent roentgenogram of her chest did not show evidence of disease.

The child was not acutely ill at the time of admission to the sanatorium and has remained asymptomatic during residence. Her weight prior to the illness was 29 lbs. (about 13.2 kg.); her weight on admission was 29¾ lbs. (about 13.5 kg.). During hospitalization she gained 2½ lbs. (about 1.1 kg.). The rectal temperature remained between 98° and 100.2° F.

Examinations of blood made the day after the patient entered the sanatorium and approximately 9 months later failed to reveal any significant abnormalities of the

\*The procedure for determination of the types of tubercle bacilli has been described previously.<sup>4</sup>

†Bronchoscopic examination was performed by Dr. E. N. Milhaft, St. Cloud, Minn.

hemoglobin or cells. On admission the blood sedimentation rate as determined by the method of Westergren was 16 mm. in 1 hour at 25° C. Nine months later the rate was 12 mm. in 1 hour at 24° C. Specimens of urine contained an increased number of leukocytes with occasional clumps, erythrocytes, and a trace of albumin. A series of cultures of urine were negative for acid-fast bacilli.

A roentgenogram of the chest on April 30, 1947, revealed marked density throughout the left upper lobe with no evident change on comparison with those taken on April 8 and April 25. The patient was tested with tuberculin on four different occasions. With the exception of the first test, both P.P.D. (purified protein derivative) and avian tuberculin were used. The results are summarized in Table I.

TABLE I  
*Results of Intradermal Injections of P.P.D. and of Avian Tuberculin*

Date	Tuberculin	Reaction*
May 5, 1947	P.P.D., 0.00002 mg.	18 by 16 mm. erythema 12 by 11 mm. induration
October 20, 1947	P.P.D., 0.00002 mg.	15 by 14 mm. erythema 13 by 10 mm. induration
	Avian tuberculin, 0.1 cc. of 1:10,000 dilution	17 by 16 mm. erythema 13 by 11 mm. induration
February 2, 1948†	P.P.D., 0.00002 mg.	18 by 17 mm. erythema 8 by 7 mm. induration
	Avian tuberculin, 0.1 cc. of 1:10,000 dilution	46 by 33 mm. erythema 27 by 25 mm. induration
May 10, 1948†	P.P.D., 0.00002 mg.	9 by 8 mm. erythema 5 by 5 mm. induration
	Avian tuberculin, 0.1 cc. of 1:10,000 dilution	28 by 22 mm. erythema 20 by 18 mm. induration

\* Recorded 48 hours after injection.

† Reactions on these dates were markedly dissimilar. The reaction to avian tuberculin was a firm, well circumscribed induration; the reaction to P.P.D. was soft, pale, and poorly outlined. The reactions to avian tuberculin persisted for several days longer than the reactions to P.P.D.

Gastric contents were collected on April 30, 1947, the day after the patient's admission to the sanatorium. Stained smears of the gastric washing were negative for acid-fast bacilli. From the portion of the material used for making cultures one colony appeared. This colony was atypical in appearance for human tubercle bacilli, but since it consisted of acid-fast bacilli a portion was used to inoculate a guinea-pig. Results of this inoculation will be referred to later in this report. Gastric contents collected on two occasions in June, 1947, and again in September and in October of the same year all failed to yield acid-fast organisms. An additional gastric lavage specimen was obtained in May, 1948. From a portion of this material attempts were made to isolate acid-fast bacilli and a portion was used to inoculate one rabbit. The results failed to reveal the presence of tubercle bacilli.\*

Since the patient was hospitalized, there has been a rapid progressive improvement of the pulmonary lesion as shown by successive roentgenograms of the chest (Figs. 1a and b). The appearance of the lesion on admission and the subsequent changes do not differ significantly from the primary tuberculous involvement found in roentgenograms of children infected by tubercle bacilli of the human type except for the absence of demonstrable calcification, which not infrequently becomes evident within

\* These procedures were done at Glen Lake Sanatorium, Oak Terrace, Minn.



a year after the onset of the disease. The patient's tonsils were still markedly enlarged when this paper was written.\*

### CLINICAL BACTERIOLOGIC OBSERVATIONS

The culture obtained from the gastric lavage specimen appeared on Petraghani's medium after 21 days of incubation. It consisted of one small colony, which had a buff color, was smooth, entire, and of a soft or pasty consistency. Considered physically unlike typical *Mycobacterium tuberculosis hominis*, the culture was tested for pathogenicity by injecting one guinea-pig intraperitoneally. When the animal died 39 days later, the spleen was enlarged but without grossly visible lesions. The liver contained one raised, buff-colored lesion. One substernal abscess was noted. Ascites and hydrothorax were recorded also. A smear preparation from the substernal abscess revealed many acid-fast bacilli. From the substernal abscess and from the spleen of the guinea-pig, cultures of acid-fast bacilli were recovered. The organism grew luxuriantly and produced a soft, smooth, unctuous growth that was similar in appearance to the original colony obtained from the gastric lavage specimen.

Histopathologic examination of the spleen of the guinea-pig inoculated with the culture from the gastric lavage specimen revealed a widely disseminated tuberculous process with numerous small tubercles situated largely in the pulp spaces. Acid-fast bacilli, while present, were few. The liver, likewise, contained similar small tubercles in addition to the larger subcapsular lesion. The latter was characterized by a necrotic central portion and a broad peripheral zone of epithelioid cells. Acid-fast bacillary forms were exceedingly numerous in the cellular debris in the center of the nodule.

After the first gastric specimen had yielded acid-fast bacilli, several additional gastric lavage specimens were examined but no additional acid-fast bacilli could be demonstrated either by culture or by animal inoculation. A summary of the attempts to demonstrate tubercle bacilli in the gastric lavage specimens is given in Table II.

The cultural characteristics of the organism obtained by gastric lavage were dissimilar in many important respects from those of tubercle bacilli of the human type. The former grew more rapidly, usually producing a luxuriant growth in 12 to 14 days. Instead of a dry, wrinkled, crumbly growth like that of the human type, cultures from the patient produced a diffuse spreading growth with discrete hemispheric colonies in the midst of the main surface growth.

\* Tonsillectomy was done on February 22, 1949, by Dr. C. R. Myre, Paynesville, Minn. It was not possible to demonstrate tubercle bacilli in the tonsils by appropriate animal inoculation or cultural procedures. Histopathologic examination revealed no acid-fast rods nor any lesions resembling tuberculosis.

The miscibility of the bacteria in distilled water was high. A uniformly turbid suspension of the bacterial cells could be obtained without diffi-

TABLE II

*Summary of Attempts to Demonstrate Tubercle Bacilli by Culture or by Animal Inoculation in Gastric Lavage Materials Collected on Six Different Occasions*

Specimen	Date	Procedure	Results
1	4-30-47	Culture	One colony of acid-fast bacilli
		Guinea-pig	Died in 39 days; tuberculous lesions of liver and spleen
2	6-11-47	Culture	Negative
3	6-14-47	Culture	Negative
4	9-26-47	Respective specimens injected on date collected into the same 2 animals, 1 guinea-pig, and 1 rabbit	Negative for tuberculosis at necropsy; cultures from spleens of both animals negative for tubercle bacilli
5	9-29-47		
6	10-3-47		
7	5-19-48	Culture	Negative
8	5-20-48	Culture	Negative
		Rabbit	Negative

culty, whereas with the usual cultures of tubercle bacilli of human type it is not easy to obtain an even suspension, clumps being eliminated with difficulty.

The growth studied had a soft, moist, and somewhat creamy consistency. It was whitish gray and had a glistening appearance by reflected light.

In liquid mediums, such as Proskauer and Beck's\* to which blood serum had been added, growth was rapid and diffuse.

The sensitivity of the culture to streptomycin *in vitro* was determined. It was found that growth was inhibited in the presence of 10  $\mu$ g. of streptomycin per cc. of medium but not by 5  $\mu$ g. This observation is perhaps of some significance, since practically all strains of tubercle bacilli of human type, isolated from patients who have not been treated with streptomycin, have sensitivity *in vitro* to less than 1  $\mu$ g. of streptomycin per cc. Conversely, the several strains of avian tubercle bacilli that we have studied have been resistant *in vitro* to at least 5  $\mu$ g. of streptomycin per cc.

The clinical and laboratory findings indicated definitely that the patient had tuberculosis but the physical properties of the one culture obtained and the atypical character of the disease produced in the guinea-pig created uncertainty as to the true character of the myco-

\*Proskauer, B., and Beck, M. Beiträge zur Ernährungsphysiologie des Tuberkelbacillus. *Ztschr. f. Hyg. u. Infektionskrankh., Leipz.*, 1894, 18, 128-152.

bacterium isolated. Consequently, additional studies were undertaken to "type" the organism by the tests of pathogenicity.\*

### EXPERIMENTAL OBSERVATIONS

*Tests of Virulence.* The inocula used for determination of the type of the infective mycobacteria were the original culture obtained from the first gastric lavage specimen and the culture recovered from the spleen of the one guinea-pig. In addition, a suspension prepared by pooling splenic materials obtained from 2 rabbits that died after inoculation with the culture recovered from the spleen of the guinea-pig was used to inject guinea-pigs, rabbits, and chickens. Four separate experiments to determine virulence were done. Pathogenicity of the bacterial culture for guinea-pigs, rabbits, and chickens was determined.† The guinea-pigs were inoculated subcutaneously, the rabbits and chickens intravenously.

The results of the tests of virulence, summarized in Table III, provide convincing evidence that the acid-fast organisms constituting the respective inocula were avian type tubercle bacilli. The lethal potentialities for rabbits and chickens were demonstrated repeatedly in an impressive manner while portions of the same inocula were essentially nonpathogenic for guinea-pigs, which were kept under observation for from 3 months to more than 4 months after being inoculated.

The fact that the one guinea-pig inoculated with a portion of the original culture obtained from the first gastric lavage specimen died presumably of tuberculosis justifies brief comment. While it is generally true that guinea-pigs have a high resistance to avian tubercle bacilli, occasionally an animal may be encountered that is markedly susceptible.‡ When this occurs, it is necessary to restudy the virulence of the infective agent by the inoculation of additional guinea-pigs. Generally speaking, mycobacteria that produce progressive and eventually severe tuberculous disease in chickens and rabbits are not capable of producing well marked or generalized tuberculosis in guinea-pigs. This is the essential difference that distinguishes avian tubercle bacilli from the human and bovine types when tests of virulence are utilized for typing.

Subsequent observations are adequate to establish that the development of tuberculosis in the one guinea-pig inoculated with the original gastric culture took place because of the unusually low resistance of this

\* Studies to determine the bacillary type of the organism obtained from the gastric lavage specimens were done in the Division of Experimental Medicine, Mayo Foundation, Rochester, Minn.

† Before being inoculated, all chickens were tested with tuberculin and failed to react.

‡ A comprehensive review of the susceptibility of guinea-pigs to avian tubercle bacilli has been published previously.<sup>1</sup>

TABLE III

Results of Tests of Pathogenicity of Acid-fast Bacteria Isolated from Gastric Lavage Specimen and of Culture Obtained from Spleen of Guinea-Pig that Died 39 Days After Inoculation with Culture Obtained from First Gastric Lavage

Experiment	Inoculation materials	Animal	Days before death	Severity of tuberculosis*			Tubercle bacilli recovered
				Spleen	Liver	Lungs	
1	Original culture from gastric lavage	Guinea-pig 1	111 (killed)	No lesions	No lesions	No lesions	From spleen
		Guinea-pig 2	111 (killed)	No lesions	No lesions	No lesions	No growth from spleen
		Rabbit 1	5 (failure)	No lesions	No lesions	No lesions	No attempt
		Rabbit 2	24	++++	++++	++	From spleen
2	Culture from spleen of guinea-pig inoculated with culture from gastric lavage	Guinea-pig 3	6 (failure)	No lesions	No lesions	No lesions	No attempt
		Guinea-pig 4	12 (failure)	No lesions	No lesions	No lesions	No attempt
		Rabbit 3	17	++++	++++	+	From spleen
		Rabbit 4	17	++++	++++	+	From spleen
3	Pooled splenic suspensions of rabbits 3 and 4	Guinea-pig 5	92 (killed)	No lesions	No lesions	No lesions	No attempt
		Guinea-pig 6	92 (killed)	No lesions	No lesions	No lesions	No growth from spleen
		Rabbit 5	24	++++	++++	++	From spleen
		Rabbit 6	29	++++	++++	+++	From spleen
		Chicken 1	61	++++	++++	+	From spleen
		Chicken 2	74	++++	++++	+	From spleen
		Guinea-pig 7	130 (killed)	No lesions	No lesions	No lesions	No attempt
		Guinea-pig 8	130 (killed)	No lesions	No lesions	No lesions	No attempt
4	Transplant of culture from gastric lavage (5 months after experiments 1 and 2)	Rabbit 7	7 (failure)	No lesions	No lesions	No lesions	From spleen
		Rabbit 8	21	++	++++	+	From spleen
		Chicken 3	33	++++	++++	No lesions	From spleen
		Chicken 4	33	++++	++++	No lesions	From spleen
		Chicken 5	32	++++	++++	No lesions	From spleen

\* Based on histopathologic examination of the tissues. Severity of infection graded 1 plus to 4 plus.

particular animal to avian tubercle bacilli. Reference to Table III shows that in 4 additional guinea-pigs inoculated subsequently with the same culture, tuberculosis failed to develop; yet portions of the same inoculum proved highly virulent for rabbits and for chickens.

*Sensitivity to Tuberculin.* All of the animals used in the typing experiments were tested with avian tuberculin and with mammalian tuberculin injected intracutaneously. Standard avian tuberculin supplied by the Bureau of Animal Industry of the United States Department of Agriculture was used. The mammalian tuberculin used was O.T. diluted 1:10. The respective dose for each tuberculin was 0.1 to 0.2 cc. All animals without exception reacted definitely and often dramatically to avian tuberculin while the reactions to mammalian tuberculin were negative or indefinite.

#### POSSIBLE SOURCE OF INFECTION

After the avian character of the culture obtained from the gastric lavage specimen had been established, a survey was made to determine the possible source of the patient's infection. A visit to the child's home revealed the usual domesticated farm animals including chickens, swine, and cattle. Arrangements were made to have all animals on the premises tested with avian tuberculin and with mammalian tuberculin.\* The results are summarized in Table IV.

TABLE IV  
*Tuberculin Tests of Farm Animals*

Species	Number tested	Tuberculin sensitivity	
		Avian	Mammalian
Chickens	430	217 (50 per cent)	2* (0.5 per cent)
Swine	6	2 (33 per cent)	None
Cattle	33	10 (30 per cent)	None

\* The same birds were also sensitive to avian tuberculin.

It can be noted that a large population of chickens was maintained on the premises and that approximately 50 per cent gave a positive reaction to avian tuberculin. (Subsequently 13 of the chickens that reacted to tuberculin were killed for necropsy and tuberculous lesions were demonstrated grossly in 11.) The fact that except during the winter months the chickens were not confined but roamed at will over the barnyard

\* The tuberculin tests were conducted by Dr. M. R. Zinober of the United States Department of Agriculture.

probably accounts for the sensitivity to avian tuberculin of 2 of the 6 swine and 10 of the 33 cattle.

The evidence obtained from this phase of the study indicated that a considerable reservoir of potential infection existed and that dissemination of the infection to heterogeneous species had taken place. That the patient associated with the chickens during playtime and assisted in cleaning and crating eggs for market provided further opportunity for exposure to the infective agent. The patient was a "thumb sucker" and this also should be considered in explaining the transmission of the infective agent. The facts definitely indicate that there existed in the child's environment sufficient opportunity for a susceptible host to become infected with the organism of avian tuberculosis.

#### COMMENT

In evaluating the evidence that accumulated in the study of this case one may summarize first those facts in support of the patient's being infected with *Myco. tuberculosis avium*. The patient did have tuberculosis. This was established clinically and bacteriologically. Every effort failed to incriminate the human or the bovine type of the tubercle bacilli. While there was no known source for infection with the mammalian type of the organism, there did exist ample opportunity for infection with the organism of avian tuberculosis.

The patient was equally or more sensitive to avian tuberculin than she was to mammalian (P.P.D.) tuberculin. The marked sensitivity to avian tuberculin is of uncertain significance, since it is well known that there exists a certain reciprocal relationship between the tuberculin sensitivity produced by avian and by mammalian types of tubercle bacilli. However, it is generally true that a more pronounced reaction is elicited by homologous than by heterologous tuberculins. In other words, there is a relative specificity to the sensitivity produced by avian and by mammalian tubercle bacilli, respectively, but a certain overlapping may be expected in some species. Considered alone, the sensitivity of human beings to avian tuberculin is certainly insufficient to establish a diagnosis of infection with avian tubercle bacilli. However, a definitely positive reaction to avian tuberculin equal or superior to the reaction to mammalian tuberculin is perhaps of some significance, especially when considered along with other and more direct evidence such as the isolation of avian tubercle bacilli from clinical material.

Another fact in support of the thesis that the patient's tuberculosis was due to the avian type of tubercle bacilli is that the culture was ob-

tained by gastric lavage 3 weeks after she had been removed from the infective environment. The period of separation from the premises where avian tuberculosis was rampant should preclude the likelihood that the tubercle bacilli obtained from the patient might have been ingested a few hours previously and represented an incidental finding.

It is also important that in the laboratory where the isolation of the organism was accomplished there was an absence of known cultures of the avian variety of tubercle bacilli.\* Therefore the occurrence of this particular culture cannot be explained by an inadvertency.

Lastly, and of most importance in support of a diagnosis of infection due to the bacillus of avian tuberculosis, is the fact that the culture of acid-fast bacilli obtained from the gastric lavage specimen was proved by adequate tests of pathogenicity to be *Myco. tuberculosis avium*. Thus we have a case, clinically and roentgenographically considered to be tuberculosis, from which was obtained from a gastric lavage specimen a culture of acid-fast bacilli. The physical appearance and pathogenesis of this organism were unlike those of either human or bovine tubercle bacilli. Instead, the organism had all the essential physical characteristics of the bacillus of avian tuberculosis and was established as such by numerous tests of pathogenicity.

Since progressive infections due to avian tubercle bacilli are extremely rare in human beings, the evidence must be considered objectively and skepticism voiced if justified. Although the evidence, as set forth in previous paragraphs, impressively supports the conclusion that the patient studied had a severe progressive pulmonary infection due to the avian variety of tubercle bacilli, a basis for skepticism does exist.

If the patient did in fact receive her tuberculous infection from the environment of the farm premises, it should be noted that the other children in the family did not become infected. Can this be explained on the basis that the patient had a greater opportunity to become infected through more frequent and intimate contact or on the basis of a peculiar intrinsic susceptibility? The true explanation must remain obscure.

Another fact that justifies skepticism concerns the failure to obtain more than one positive culture from the patient. It will be recalled that only a single colony of acid-fast bacteria was obtained. In animal infections due to avian tubercle bacilli the organisms are usually exceedingly numerous and can be cultured readily. In this instance, although several subsequent attempts were made to culture tubercle bacilli from gastric lavage specimens, the second attempt was not made

\* The organism was isolated in the bacteriologic laboratory of Glen Lake Sanatorium.

until 42 days had elapsed. During this interval the patient had been kept on strict sanatorium regimen. Perhaps the progress of the disease had ceased and regression had begun. If healing and resolution did commence during this period, an influence on the number of tubercle bacilli finding egress from the diseased lung might be expected.

Lastly, the fact that the original culture proved virulent for the one guinea-pig inoculated was indeed unusual. This can be dismissed by again pointing out that an occasional guinea-pig has an exceedingly low resistance to avian tubercle bacilli. In this instance subsequent studies of the culture established the fact that it was essentially nonpathogenic for guinea-pigs.

Considering all of the evidence one may conclude that (1) the patient did have tuberculosis and (2) the causative organism was probably *Myco. tuberculosis avium*. However, the evidence necessary for the absolute proof of the etiologic relationship of this variety of the tubercle bacillus to the patient's illness was not obtained. Perhaps subsequent observations of the patient may provide data that will justify more definite conclusions.

#### SUMMARY

A study was made of a culture of acid-fast bacilli from a gastric lavage specimen obtained from a female child approximately 2½ years of age. Roentgenographically the lungs showed extensive involvement of the left upper lobe diagnosed as tuberculosis. The child had lived on a Minnesota farm on which tuberculosis among the chickens was rampant. The child played with the chickens, handled eggs, and was a "thumb sucker." Extensive studies of the culture obtained revealed that the physical characteristics and pathogenic behavior of the culture were similar in every respect to those of *Mycobacterium tuberculosis avium*. While the causative relationship of the bacterium to the pathogenesis of the pulmonary disease could not be proved definitely, the evidence strongly indicates such a relationship. The study illustrates the difficulties of proving unequivocally the etiologic relationship of *Myco. tuberculosis avium* to tuberculous infections in human beings.

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## DESCRIPTION OF PLATE

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### PLATE 183

FIG. 1*a*. Chest of patient at time of admission to sanatorium. There is extensive infiltration of the left lung. *b*. Chest of patient 9 months later. Marked improvement in the appearance of the left lung is evident.



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# LYMPHOID TISSUE AND ITS RELATION TO SO-CALLED NORMAL LYMPHOID FOCI AND TO LYMPHOMATOSIS

## I. QUALITATIVE STUDY OF LYMPHOID AREAS IN THE PANCREAS OF CHICKENS\*

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Birds, generally, are known to possess numerous, widely scattered accumulations of lymphoid tissues in various organs. Danchakoff<sup>1</sup> and Mjassojedoff<sup>2</sup> in their studies on hematopoiesis regarded these structures as normal to the bird. They are said to correspond to the lymph nodes of mammals.<sup>3,4</sup> Nonidez<sup>5</sup> described the development of eosinophils in the region of the gonad. Jordan<sup>6</sup> has described granulopoietic transformation of cells from lymphoid nodes located in the bone marrow, liver, and other organs of birds. The fact that accumulations of lymphocytes do show potentialities of development into cells of other types may not be adequate evidence that the region where they are found should normally have lymphoid tissue. The term "normal" is used here in the sense that normal structures arise as a part of the ontogenetic processes of the bird in anticipation of future needs. Examples of lymphoid structures which may be regarded as normal are the thymus, bursa of Fabricius, and spleen. If the lymphoid tissue in parenchymal organs has developed in response to external agents, it is called abnormal in this study. The term "abnormal" in no wise precludes the possibility that these lymphoid areas may be valuable to the general well-being of the host in defense against foreign external agents, and that they may not serve hematopoietic functions. The terms have been used to simplify the discussion of the problem. It should be added that a descriptive embryologic study alone will not give us the answer in view of the numerous suggestions made in the literature that the agent of lymphomatosis, as well as certain bacteria, is transmitted through the egg.<sup>7-9</sup>

Lymphomatosis is a disease involving the lymphoid tissue of the body and in many cases the lesions are found in the same organs which contain the lymphoid foci, the normality of which is being questioned. Numerous publications have described the clinical symptoms and pathologic alterations which seem to be involved in the avian leukosis complex.<sup>7,10-14</sup> Lymphomatosis is one aspect of the complex, and in the material involved in this series of papers is represented by the ocular, neural, visceral, and osteopetrotic forms.

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† United States Department of Agriculture, Agricultural Research Administration, Bureau of Animal Industry.

A criterion for diagnosis of this disease is the presence of grossly visible tumors at necropsy. The tumors among different individuals vary in size from a barely perceptible swelling on a single nerve or organ to large, overgrown, multiple tumors. It is conceivable that large, proliferating tumors may be a direct cause of death but it is hard to believe that a barely visible tumor which apparently does not interfere with a vital function can be the immediate and direct cause of death. According to Doyle,<sup>10</sup> "The pathology of this disease . . . strongly suggests that it is infectious in nature." If lymphomatosis is an infectious disease, then its causative agent may well produce systemic effects on the organism in addition to stimulative effects on lymphoid tissue. The latter under suitable conditions may produce a neoplastic, transplantable tumor as an end result.

The purposes of this series of papers are to present qualitative and quantitative evidence which can be brought to bear on the problems of normality of ectopic lymphoid foci, to determine the significance of these lymphoid reactions in relation to lymphomatosis and, finally, to give some suggestions concerning the microscopic diagnosis of the disease. The present paper deals with descriptive aspects of the problem.

The pancreas rather than the liver was chosen for the initial studies for several reasons: (1) The lymphoid areas are usually sufficiently focal to be easily measured; (2) the pancreas is not considered a normal hematopoietic organ, either in birds or in mammals; (3) its slender shape and uniformity of internal organization facilitate the taking of comparable sections; and (4) the same slides can be used for a comparison between intranuclear inclusions present in the islands of Langerhans and the lymphoid tissue. Later studies will be extended to other species and other organs.

It goes without saying that an adequate diagnosis of the bird as a whole cannot be made on one section. The pancreas is merely one of a group of organs and tissues which might be chosen for study. However, by intensive study of one organ it should be possible to elucidate basic principles involved in lymphoid reactions, which should be applicable to all parts of the body.

#### MATERIALS AND METHODS

Survivors of one year's population used in the breeding program of the Laboratory formed the material for this study. The birds were killed at an average age of slightly over 600 days. All were Single-Comb White Leghorn females. The original population was composed of 1123 birds divided among 14 inbred lines and an  $F_1$  cross of two of these lines ( $9 \times 10$ ).

The incidence of grossly visible lymphoid tumors found at necropsy has been used as a measure of resistance and susceptibility. On this basis the inbred lines and cross varied from 7.5 to 77 per cent in incidence of lymphomatosis based on the original population, or from 9 to 100 per cent when the population was corrected for deaths not associated with gross tumors.

Tissues were taken from 319 of the 352 survivors of the population and from all 14 of the surviving birds which were diagnosed as grossly positive for lymphomatosis. As to the anatomic type, of these 14, 4 were neural, 8 were visceral, 1 neural and visceral, and 1 ocular. In no case was the pancreas diagnosed as positive for lymphoid tumor on gross examination. Diagnoses based on gross lesions were taken from the necropsy records compiled by the Laboratory veterinarians.

A portion of pancreas about 13 to 19 mm. long was removed from approximately the center of the organ and fixed in Petrunkevitch no. 2.<sup>20</sup> All sections were cut at 5  $\mu$  and stained with Galigher's hematoxylin and triosin.<sup>15</sup>

The data on areas of the total section and of lymphoid tissue were obtained by tracing the projected images with a planimeter and converting the reading to square millimeters. When projecting the sections, large vessels and ducts were excluded from the drawing so that values obtained were a measure only of parenchymal tissue and small vessels and ducts. The entire section was searched for lymphoid areas but only those were measured which were located below the serosal connective tissue. Thus those which formed part of the pancreatic omentum or lay at tips of lobes were not included.

#### OBSERVATIONS

The photomicrographs and drawing in Figures 1 to 18 have been prepared to show some major trends in development which may lead to a large proliferative tumor. The specific data for each figure are given in Table I. Cases have been selected to illustrate a wide range (0.1 to 88.4 per cent) of lymphoid involvement. They have been chosen also to show focal, perivascular, and diffuse lesions. In some cases both the smallest and largest lymphoid areas of the same section are shown so that the reader may judge for himself whether there exist any fundamental histologic differences between the two. Lymphoid areas from most resistant to most susceptible lines are included to bring out any differences which may be related to these variables. One drawing was made to show the details revealed by the microscope in the hope

that it might aid in the interpretation of the less distinctly seen structures shown in the photomicrographs.

A study of the 319 slides involved in this experiment showed only one case which histologically was strikingly different from all of the others. It is shown in Figures 17 and 18 and came from a section having 88.4

TABLE I  
*Data Relative to Figures 1 to 18*

Figure no.	Chicken no. (1945 population)	Gross diagnosis for lymphomatosi	Line of chickens	Per cent* of lymphomatosi in line	Per cent of lymphoid area for the section	Number of lymphoid areas in the section	Area in sq. mm. of lymphoid accumulation from which photomicrograph was taken
1	620K	—	13	44	0.1	1	0.02346
2	1280	—	3	67	0.4	5	0.00323
3	263X	—	6	9	0.4	5	0.00427
4	263X	—	6	9	0.4	5	0.05334
5	574B	V	12	100	0.8	6	0.07933
6	267T	—	6	9	1.6	8	0.10135
7	354H	—	9X10	40	1.8	25	0.00085
8	354H	—	9X10	40	1.8	25	0.08658
9	354H	—	9X10	40	1.8	25	0.00654
10	354H	—	9X10	40	1.8	25	0.00654
11	357Q	—	9X10	40	3.2	29	0.00128
12	357Q	—	9X10	40	3.2	29	0.07208
13	132A2*	—	3	67	8.8	34	0.01067
14	132A2	—	3	67	8.8	34	0.18847
15	739E	NV	15	64	29.3	19	0.01422
16	739E	NV	15	64	29.3	19	2.07848
17	321B2	O	7	33	88.4	9	16.25600
18	321B2	O	7	33	88.4	9	16.25600

N = Neural lymphomatosis.

V = Visceral lymphomatosis.

O = Ocular lymphomatosis.

— = Negative for lymphomatosis.

\* Population corrected for deaths not associated with gross tumors.

per cent of lymphoid involvement. This case was neoplastic but in the same slide certain areas were found having the histologic character of the reactions shown in the preceding figures. The remaining cases were examined to determine if there was a series of precursory reactions taking place.

### *Nuclear Size*

One generally accepted criterion of a lymphoid tumor is the presence of lymphoid cells with large nuclei and a clumping of chromatin around enlarged nucleoli. In Figures 1 and 2 (0.1 and 0.4 per cent involvement) there are a few medium-sized nuclei. They are more conspicuous in the large diffuse area (Fig. 1) than in the small perivascular area (Fig. 2). The first figure is from a more resistant line than the second one. Figures 3 and 4 are the smallest and largest foci from a case selected from the most resistant line of the Laboratory stock. The histologic character of the two is considerably different; the small area seems relatively

unreactive, whereas in the larger area there exist fibroblasts, macrophages, one mitotic figure, and destruction of adjacent tissue. Yet the medium-sized proliferative cells such as are seen in Figure 1 are absent. In Figure 5, which is taken from a case showing a relatively small amount of lymphoid tissue (0.8 per cent), there are numerous medium-sized nuclei, but it should be pointed out that this line had more lymphomatosis than any other. This bird was the only survivor of the line, and when killed showed gross visceral involvement of liver, spleen, and kidney, although the pancreas was considered negative. Only one or two nuclei of medium size can be seen in Figure 6 (1.6 per cent lymphoid tissue). This case had the largest amount of lymphoid tissue of any survivor from the most resistant line of the Laboratory flock. The area from which Figure 6 was taken was larger than that used for Figure 5.

Figures 7 to 10 are from one case of the  $9 \times 10$  cross in which the section showed 1.8 per cent involvement. The area shown in Figure 7 is the smallest and has 1 mitotic figure and perhaps 2 or 3 slightly larger nuclei but they are not equal to those shown in Figure 5. Figure 8 is from the largest lymphoid area of the section and shows several medium-sized lymphoid nuclei and abundant examples of gradation in nuclear size down to those of small lymphocytes. The lymphoid area shown in Figures 9 and 10 would be considered relatively small on the distribution curve for size of individual lymphoid areas; only 5 of the 25 foci in this section were smaller, yet it shows the same general characteristics as did the largest area of the section. In these figures there are several lymphocytes with nuclei of medium size, a few macrophages, some fibroblasts, and 5 mitotic figures. Figures 11 and 12, also from the  $9 \times 10$  cross, are the extremes in size from a case which had 3.2 per cent lymphoid tissue in the section. Medium-sized lymphoid nuclei are conspicuous in both figures.

Figures 13 and 14 illustrate the extremes in size from a case showing 8.8 per cent lymphoid tissue and 34 separate foci. The lymphoid area in the latter figure was almost 0.2 sq. mm., yet in this bird there seemed to be no greater number of medium-sized lymphoid nuclei than in Figure 2, which was taken from line 3 also but had only 0.4 per cent lymphoid tissue. The case with 8.8 per cent lymphoid tissue, however, showed clear evidence of acinar destruction (Figure 14).

Figures 15 and 16 again illustrate the extremes in size of lymphoid areas and these are from a case in which 29.3 per cent of the section was involved. The largest area was over 2 sq. mm. The case was diagnosed grossly as both neural and visceral but the pancreas was negative. Line 15 from which it comes is relatively susceptible to lymphomatosis,



yet in spite of these various favorable circumstances there were very few medium-sized lymphoid nuclei. There is, however, more nuclear degeneration (Fig. 16) than in the preceding examples and there is some tissue disorganization. However, cytologic and histologic changes in the lymphoid tissue itself are not sufficiently distinctive from other cases which have much smaller amounts of lymphoid tissue to warrant use in differentiating between a positive and negative microscopic diagnosis.

The case represented by Figures 17 and 18 has been mentioned and shows a condition which probably could be correctly classified as neoplastic. Since all photomicrographs are of the same magnification, the increase in size of the lymphoid nuclei is evident. Mitotic figures are seen in both fields and abnormal mitotic figures were found.

As to size the lymphoid nuclei have shown a predominance of small and medium types, and only in the case with maximum proliferation were large lymphoid nuclei found. Much has been written about the large lymphocyte or hemocytoblast. Is it what has been called here a medium-sized lymphoid cell or is it the very large cell type shown in Figure 18? If the presence of the latter is the criterion for microscopic diagnosis, then only 1 of 319 cases would be classified as microscopically positive and those having 2 to 30 per cent of lymphoid tissue would have to be classed as microscopically negative.

### *Type of Lesion*

Investigators working with lymphomatosis have observed various types of lymphoid lesions: (1) focal with a bounding connective tissue capsule, (2) focal without a capsule (perivascular), and (3) diffuse types. There seem to be no fundamental differences among various types and probably they represent merely the varied reaction which the lymphoid cells are capable of showing under different environmental conditions in the tissues, such as have been suggested as factors in hematopoiesis. Connective tissue acts as a natural barrier and is probably effective in restraining the migration of lymphoid cells. Numerous foci showed encapsulation by connective tissue, and examination of those which were small seemed to show a graded series of changes leading to intravascular stasis which might possibly have its origin in the sludging phenomenon of blood described by Knisely, Bloch, Eliot, and Warner.<sup>16</sup> Intravascular lymphoid reactions are common in chicken tissues but their relationship to lymphomatosis has not been satisfactorily determined. An example of such a reaction is shown in Figure 1 and may represent the initial stages in a focal tumor. The structure shown in

Figure 7 probably was an artery, but circulation stopped, or at least slowed down, so that mitosis took place. The central area in Figures 9 and 10 is surrounded by connective tissue remnants of the vascular wall which probably has been expanded by mitotic activity. Growth of lymphoid nodules by mitosis in birds has been mentioned by Jordan.<sup>6</sup> The process, once started, can continue and great expansion ultimately results (Fig. 12) so that the encapsulated area is much larger than the vessel from which it probably arose. The intravascular plug and its expansion usually are characterized by a closer aggregation of the cells than occurs in perivascular areas. Eventually the connective tissue remnants break down and the adjacent areas merge. Numerous examples of this were observed. Histologic evidence of the continuity of a focal area, such as shown in Figure 9, with the lumen of a patent vessel has not yet been observed and thus it is assumed that during development the involved vessel degenerates and becomes detached. The evidence indicates that stasis and lymphocytic development start in small vessels rather than in larger ones, but apparently not in capillaries. This may help to explain the observations made in an earlier report<sup>17</sup> that lymphoid accumulations rarely, if ever, originate in the pancreatic islets, which contain primarily a capillary circulation.

Foci which originate as perivascular areas are more loosely bound by a network of connective tissue (Figs. 2 and 3) than are the intravascular, focal areas. As they proliferate they are only partially restrained by the connective tissue (Fig. 13) and displacement of adjacent normal parenchymal tissues often has been observed.

In the diffuse type the lymphoid cells which chance to accumulate in the sparse connective tissue around the acini are apparently the least restricted in their migration (Fig. 1). This type is relatively rare in the pancreas. When it occurs it is destructive to the adjacent acinar and islet tissues. It has been observed in some cases that the encroaching focal or diffuse lymphoid tissue was destroying intrinsic ganglia of the pancreas, but never has a small focal area appeared to have its origin within a ganglion. This was surprising in view of the fact that the larger visceral ganglia are reported to have lymphoid areas.<sup>7</sup> The same explanation given for the islets is probably applicable here, that the vessels of these small intrinsic ganglia, which seldom show more than 3 or 4 cells in one cross section, are limited to capillaries.

For diagnostic purposes in determining whether a bird has lymphomatosis, there seems to be little practical value in separations based on the type of lymphoid involvement, especially since very frequently all types can be found in the same section.

## DISCUSSION AND CONCLUSIONS

Can any lymphoid accumulation in a parenchymal organ such as the pancreas be regarded as normal for the bird? It has been suggested by others<sup>1-4</sup> that such lymphoid accumulations exist because most birds do not have lymph nodes as do mammals, yet it has been pointed out in this study that some lymphoid areas arose from occluded vessels, which obstruction can hardly be regarded as normal for the vascular system; others are perivascular and sometimes cause the vessel wall to bulge into the lumen, which again can hardly be considered normal and possibly interferes with the flow of blood. Invasive lymphoid areas show histologic evidence of destruction of acinar tissue, pancreatic islets, and intrinsic nerve ganglia, as well as displacement of these tissues. Thus, histologically, there seems little evidence that any ectopic lymphoid accumulation is normal for a healthy chicken. True, its presence may possibly indicate a desirable defense reaction, but it has already been pointed out that there is a difference in the way one should regard aggregations of lymphoid tissue in organs such as the thymus, bursa, and spleen, which develop in anticipation of future needs, and lymphoid foci which arise in response to a foreign stimulus. The former has been called normal and the latter abnormal, or at least indicative of pathogenic agents at work.

It is obvious, of course, that it would be simpler to classify only those tumors showing very large lymphoid cells and wild proliferation, such as pictured in Figures 17 and 18, as microscopically positive for lymphomatosis and all other cases as microscopically negative. To do so, however, immediately raises several difficulties in that small lesions in the nervous system conventionally diagnosed as positive for lymphomatosis often do not show large lymphoid cells or a reaction suggestive of neoplasia, but the general evidence of association with other features of this disease seems to justify including the neural form in the disease complex. Likewise, one is faced with the problem of evaluating a section in which up to 29 per cent of the area is lymphoid and yet is otherwise little different histologically from sections in which the amount is small.

*It becomes extremely difficult and probably impossible on the basis of histologic evidence alone to set forth clear-cut lines of separation between hyperplastic and neoplastic reactions. In the sections studied there are all gradations from small perivascular areas, which seem to be doing no harm, up to large destructive lymphoid proliferations. There is no more basis for regarding a minimal amount of lymphoid tissue as normal than there is for considering it abnormal. The chief arguments for its normality are its widespread distribution among birds and, by*

comparison, its uncommon occurrence in mammals which have lymph nodes, but these points do not constitute factual evidence for normality in the sense in which the term "normal" has been used in this paper. Kelsall<sup>18</sup> has made the interesting observation that the presence of large mixed cell sarcomas in rats did not change the size or shape of lymphocytes from those present in the intestine of control animals. Kelsall and Crabb<sup>19</sup> later showed that the liver of hamsters, which does not normally have lymphoid areas, will develop periportal lymphocytes and plasma cell infiltrations with hematopoiesis, shortly following subpancreatic implantation of a mixed cell sarcoma.

Over 20 years ago Pappenheimer, Dunn, and Cone<sup>7</sup> were impressed by the fact that there was very little difference in the lymphoid picture of birds showing grossly visible lesions and the ones which did not. They suggested that many birds believed negative for lymphomatosis very probably were under stimulation of the agent and in their opinion microscopic diagnosis was arbitrary. However, our report presents some evidence for the abnormality of the occurrence of ectopic lymphoid areas and emphasizes that qualitatively, at least, no histologic evidence exists which precludes the possibility that even minimal amounts of lymphoid tissue are indicative of the agent responsible for lymphomatosis.

#### SUMMARY

A histologic study was made of lymphoid areas found in one section from each of 319 survivors of an original population of 1123 chickens. These represented 14 inbred lines and a cross between two of the lines.

The pancreas was grossly negative for lymphoid tumors in every case.

The amount of lymphoid tissue in the pancreas varied from none to 88.4 per cent of the section and from none to 34 separate foci. Only in the case with 88.4 per cent of lymphoid tissue was the histologic picture sufficiently distinctive to separate it clearly from the other cases. Throughout the range from amounts of less than 0.1 per cent to 30 per cent there were no distinctive histologic criteria which, independent of quantity, could form a basis for diagnosis. Nor did resistance, susceptibility, hybridization, or inbreeding appear to be regulating factors in the histologic picture.

Focal areas bounded by connective tissue were interpreted as derived from plugged and expanded blood vessels. It was suggested that the diffuse and invasive character of the lesions was influenced, at least in part, by the connective tissue matrix of the lesion and that, on that basis, the type of lesion had no great value in arriving at a diagnosis

of lymphomatosis, although some types of lymphoid lesions may be more damaging than others.

Consideration was given to the problem of the normality of any lymphoid foci in the pancreas and, in view of the numerous examples of destructive action of these lymphoid areas, the normality of all of them is questioned. Moreover, a review of evidence in the literature reveals no convincing basis for their normality.

Acknowledgment is made to Janet B. Breitmayer for technical assistance in this study.

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[ *Illustrations follow* ]

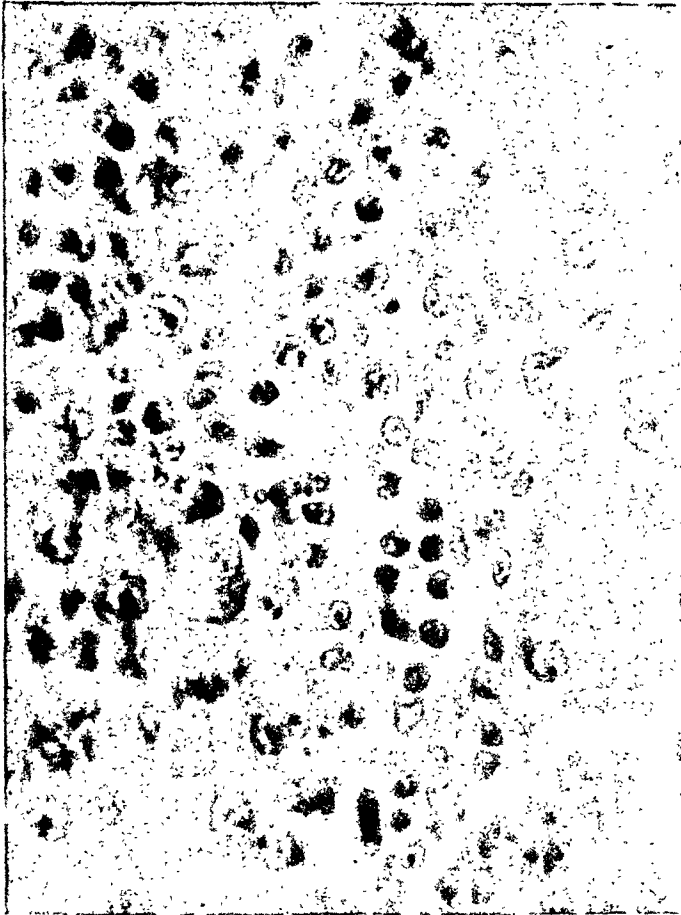
## DESCRIPTION OF PLATES

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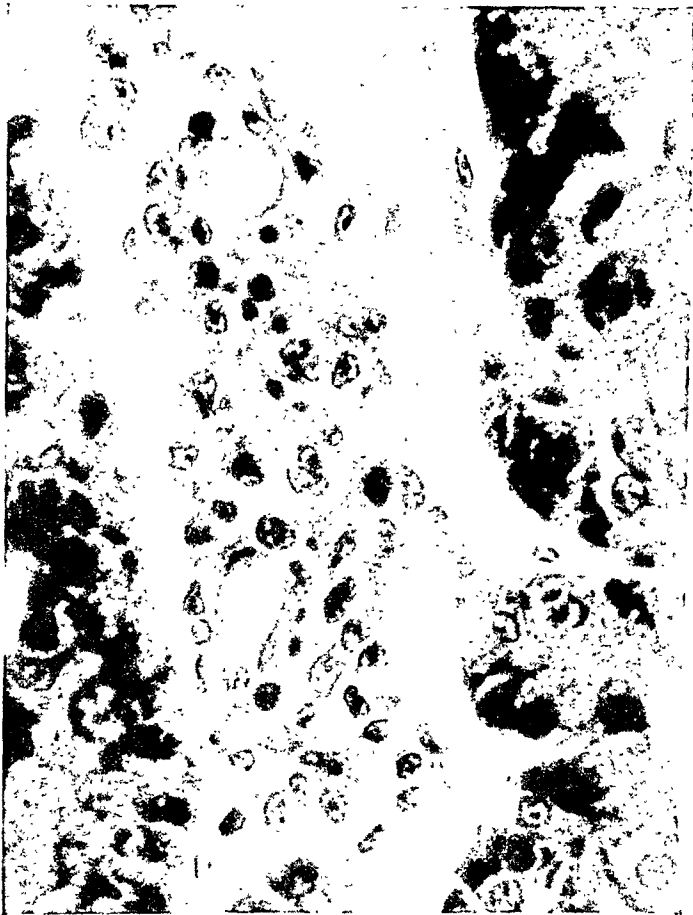
### PLATES 184-188

Figures 1 to 18 are presented to show the histopathologic variations in representative lymphoid areas. The photomicrographs were taken at an optical magnification of 762 times and the drawing was made at an optical magnification of 645 times. Magnification for publication is 639  $\times$  for all figures except Figure 10 which is 941  $\times$ . The magnification of Figure 9 after reduction is 516  $\times$ .

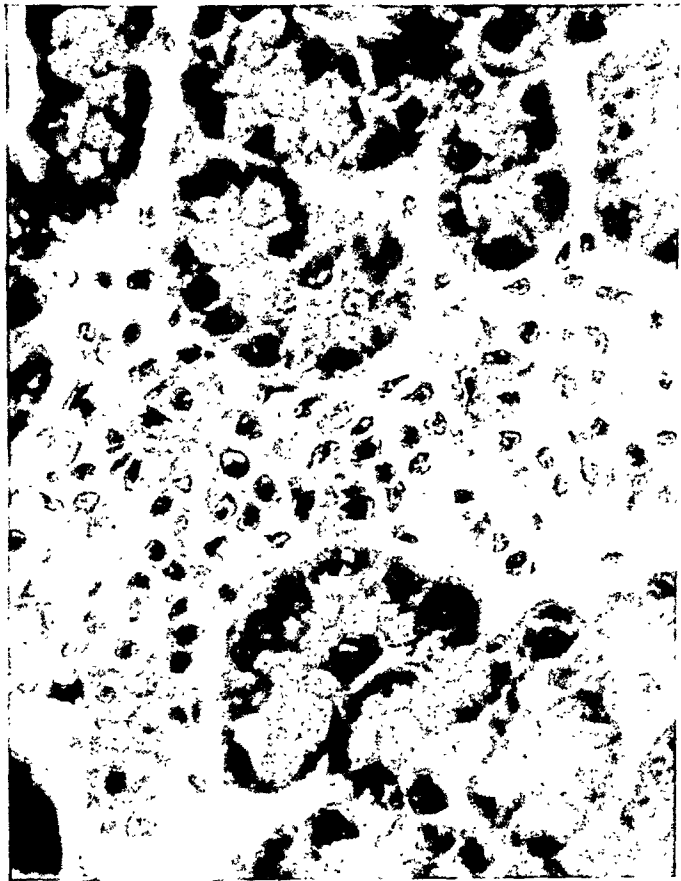
The salient data concerning each figure have been presented in Table I in the text.



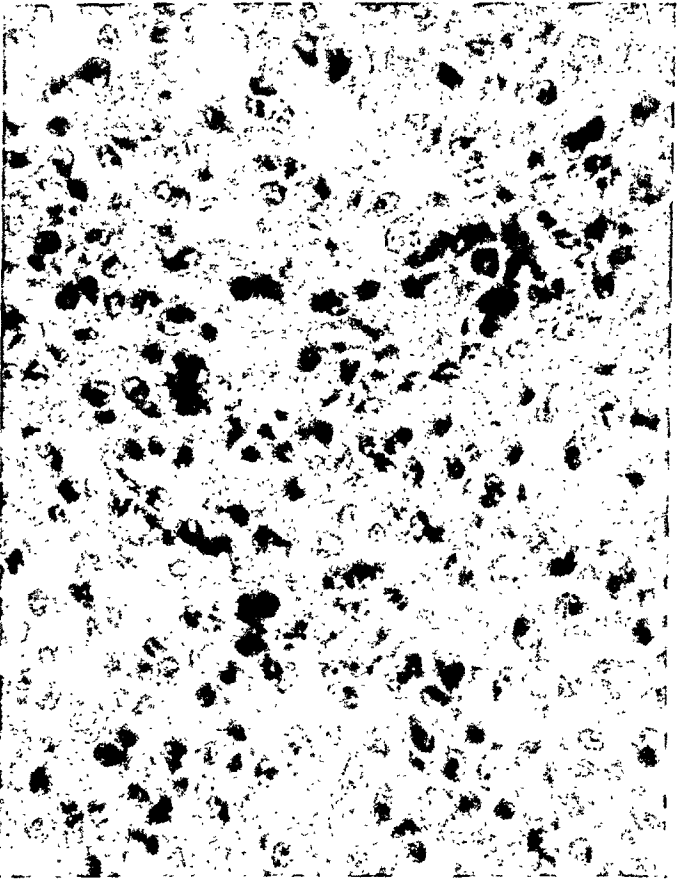
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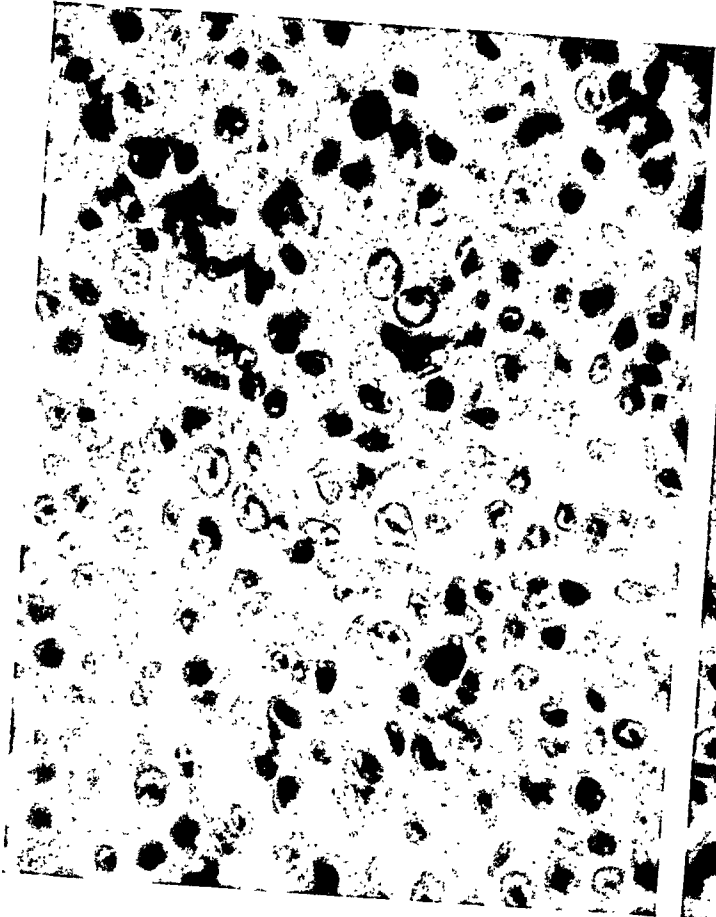


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Lucas

Lymphoid Areas in the Pancreas

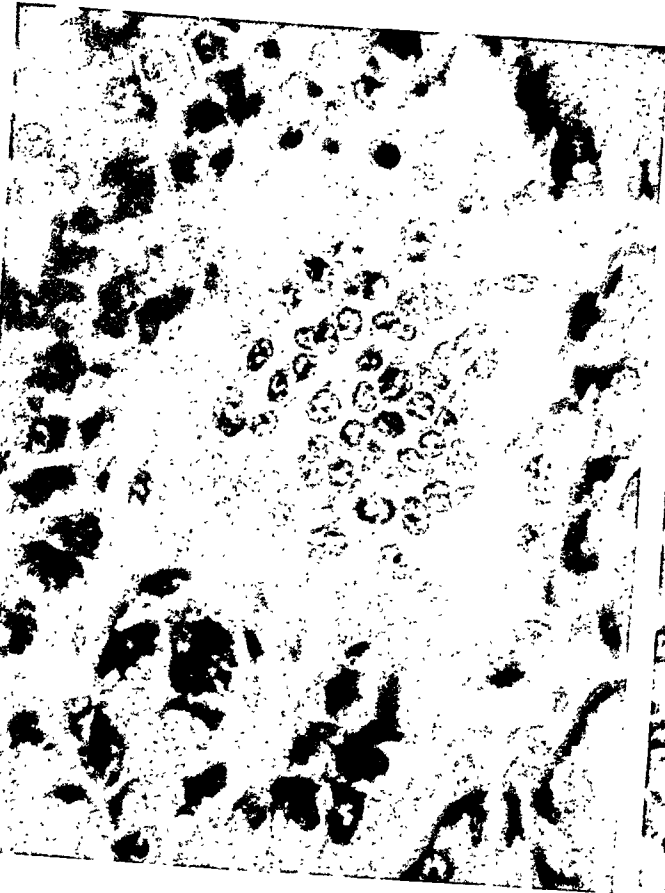




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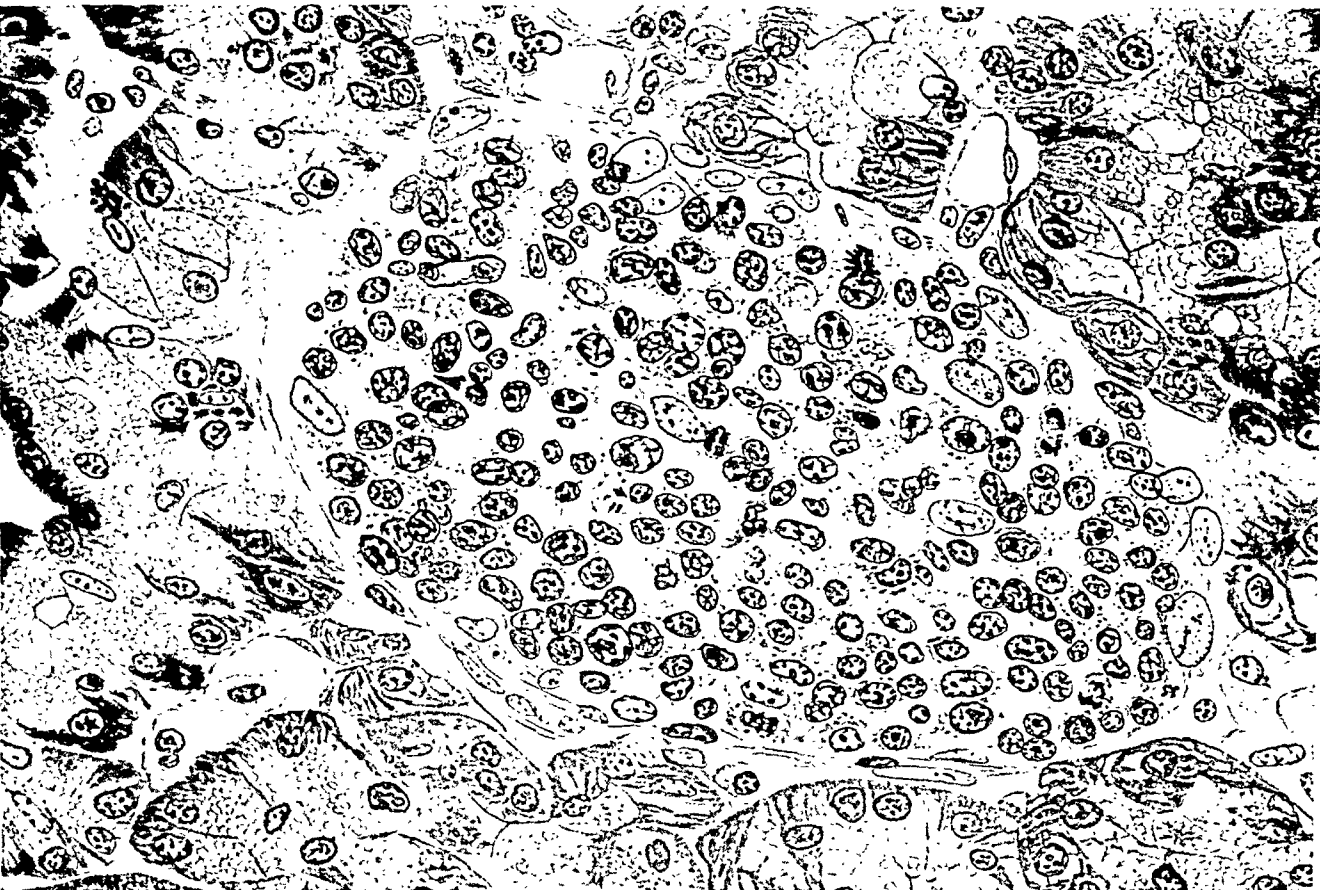


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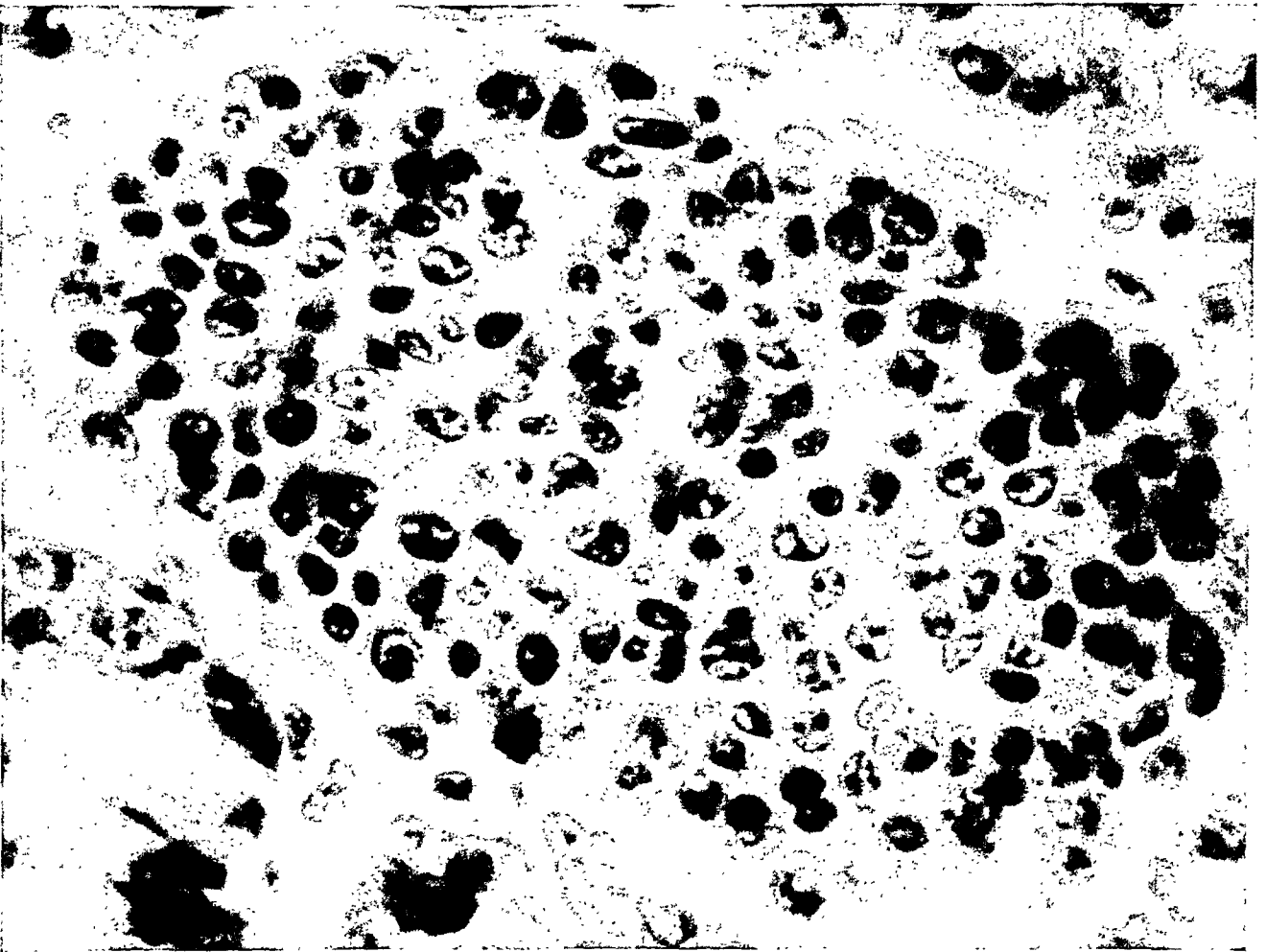


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Lymphoid Areas in the Pancreas



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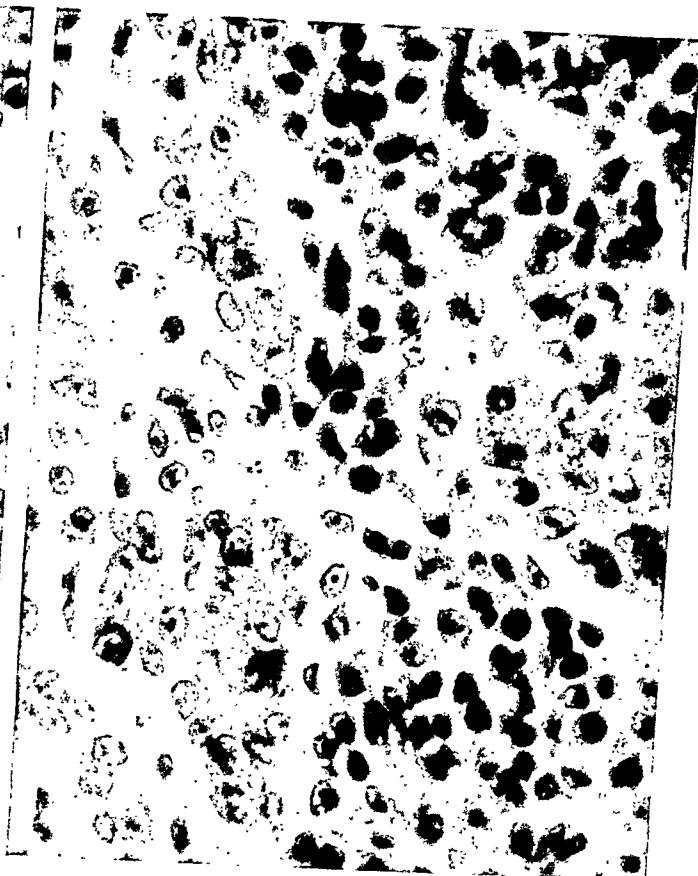
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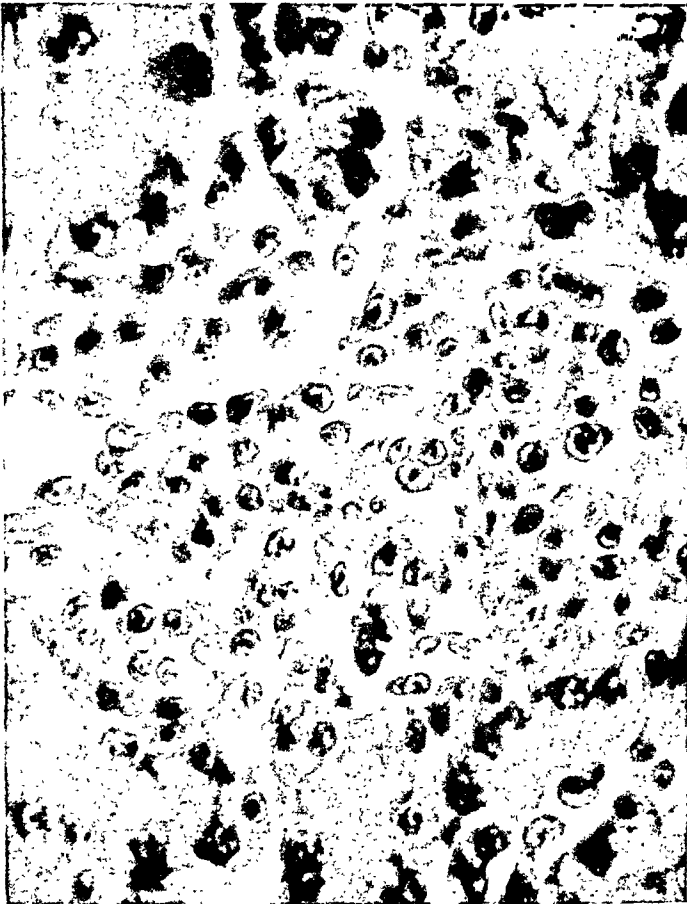
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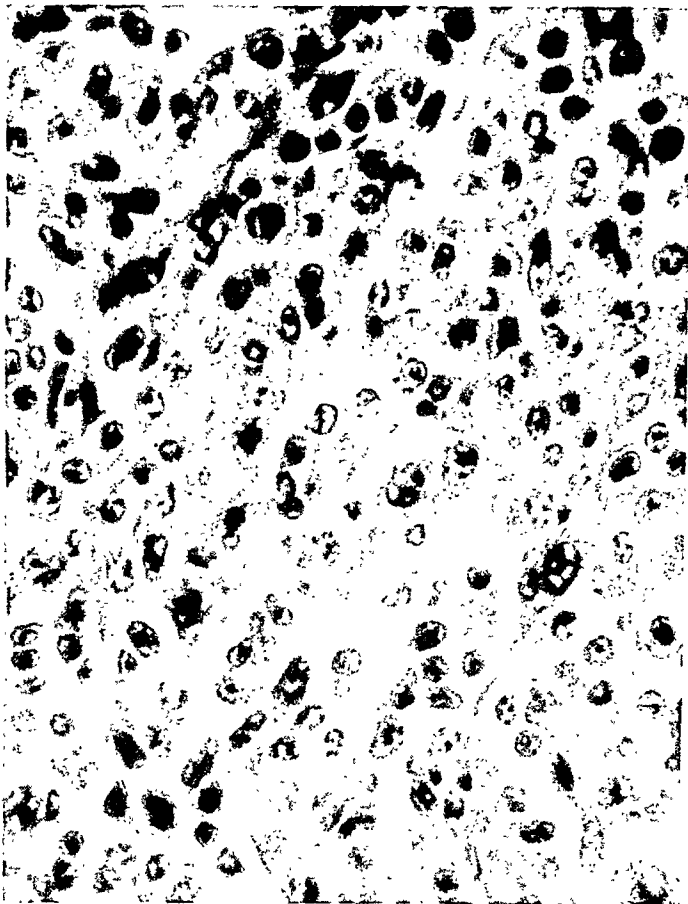
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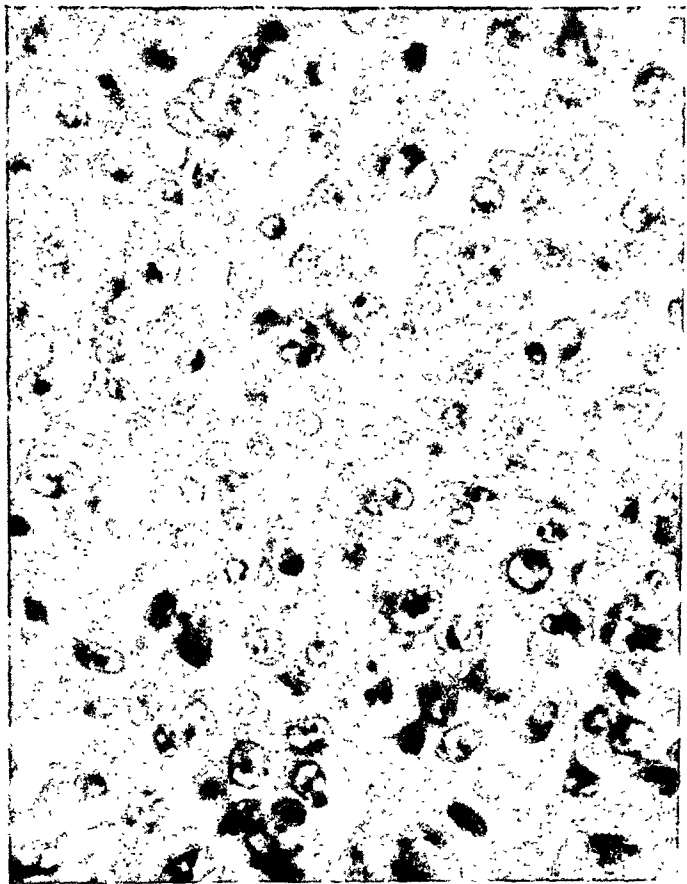
Lymphoid Areas in the Pancreas



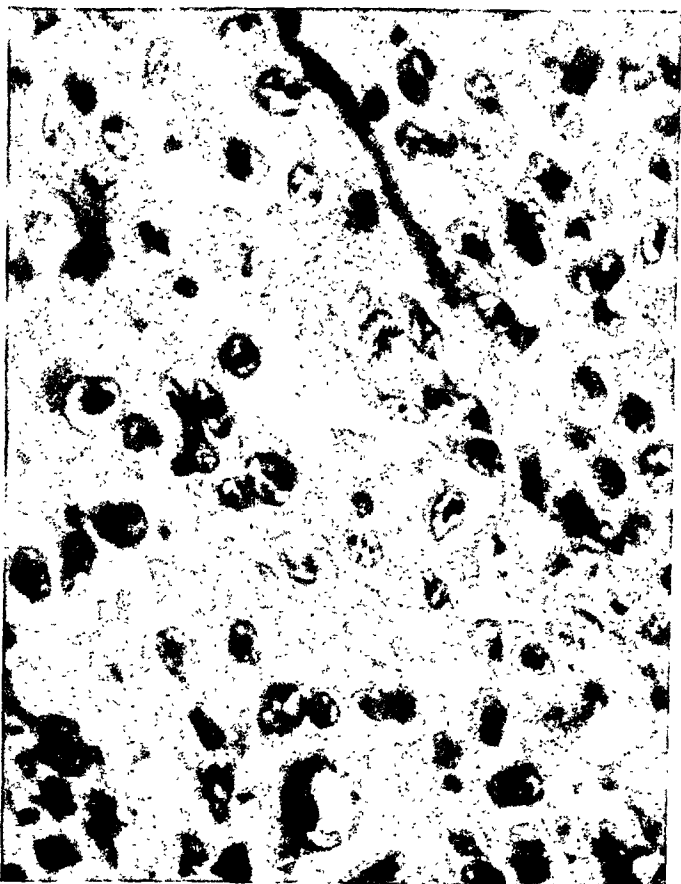
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Lucas

Lymphoid Areas in the Pancreas



# SPONTANEOUS CARDIAC LESIONS IN MICE THEIR BEARING ON ATTEMPTS TO PRODUCE EXPERIMENTAL CARDITIS\*

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Following the method of multiple parenteral injection of foreign protein used by Rich and Gregory<sup>1-3</sup> and others<sup>4-6</sup> to produce lesions of rheumatic and periarteritic type associated with anaphylactic hypersensitivity, Moore and his co-workers,<sup>7</sup> in 1947, reported the occurrence of "rheumatic-like" cardiac lesions in mice given multiple parenteral injections of diluted egg white. They stated that all their mice, whether sensitized by the intravenous or intraperitoneal route, developed lesions resembling those seen in acute rheumatic carditis in man and those described by Rich and Gregory<sup>2,3</sup> in rabbits. They pointed out that some untreated mice had minimal lesions which they called "rheumatic stigmata" but that these lesions were neither as frequent nor as marked as those in treated animals.

The present report deals with an attempt to repeat the work of Moore and his associates.<sup>7</sup> It was found that normal mice showed spontaneous cardiac lesions similar to those reported in their treated mice. The incidence and severity of the spontaneous cardiac lesions reported here were not increased by multiple parenteral injections of diluted egg white.

## MATERIALS AND METHODS

Egg white, separated from the yolks of 1-day-old hens' eggs, was beaten to break up the mucin sacs, filtered through several layers of gauze, diluted with nine parts of sterile distilled water and then refiltered to remove the precipitated mucin. Each of the mice inoculated intraperitoneally received 1 cc., and each of the mice inoculated intravenously received 0.2 cc. of diluted egg white prepared immediately before injection. All mice treated with multiple injections received them at weekly intervals.

The "typhoid free" white Swiss mice, 3 weeks of age, used in the experiments, were all obtained on the same day from one dealer so that treated mice and controls were comparable as to source, age, diet, and housing conditions. Mice killed or dying as a result of inoculation were immediately autopsied and the organs fixed in formalin. The hearts were semi-serially sectioned at levels which would permit observation

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† Helen Hay Whitney Foundation Fellow.

of all valves. From 10 to 40 sections of each heart, stained with hematoxylin and eosin, were examined microscopically.

### EXPERIMENTAL FINDINGS

In the attempt to produce cardiac lesions in mice made hypersensitive to diluted (1:10) egg white, 145 mice were used. One hundred were given multiple parenteral injections and 45 mice were used as controls.

Initially, each of the 100 treated mice received three weekly intraperitoneal injections of freshly prepared egg white and the 96 survivors were divided and variously treated so that at the conclusion of the experiments the hearts of five groups of mice treated in different ways (Table I) were sectioned: Group A, those surviving four intraperitoneal

TABLE I  
*Number of Mice and Variation in Treatment*

	Number treated	Route and number of injections	Number examined microscopically
Group A	23	4 I.P.*	21
Group B	26	3 to 11 I.P.	19
Group C	15	3 I.P. and 1 I.V.†	13
Group D	32	3 I.P. and 1 to 5 I.V.	30
Group E	4	11 I.P. and 1 to 3 I.V.	3
Total treated	100		86
Group F	25	1 I.V.	19
Group G	20	None	20
Total controls	45		39

\* I.P. = intraperitoneal.

† I.V. = intravenous.

injections and killed at weekly intervals without further injections; group B, those dying of anaphylactic shock as a result of the third or subsequent weekly intraperitoneal injections (up to nine doses); group C, those surviving three intraperitoneal and one intravenous injections and thereafter killed at weekly intervals; group D, those surviving three intraperitoneal and one intravenous injections but dying of anaphylactic shock as a result of subsequent intravenous injections; group E, 4 mice surviving eleven intraperitoneal injections and dying of anaphylactic shock after subsequent intravenous injections.

Anaphylactic hypersensitivity to diluted egg white was produced frequently as evidenced by death due to anaphylactic shock occurring within  $\frac{1}{2}$  hour after inoculation. There was a progressive increment of anaphylactic deaths with each successive injection after the third, the increment being greater with the intravenous than with the intraperitoneal route.

Two groups of control mice were used in the experiments. The first, group F (25 mice), received a single intravenous injection of diluted egg white on the same day that mice treated with multiple injections received their fourth dose. The second, group G (20 mice), were untreated. The mice in each group were killed and autopsied at weekly intervals so that the ages in the control groups were comparable to those in all of the treated groups.

Eleven mice found dead from 2 to 18 hours after an injection and 9 mice (3 treated and 6 controls) found dead at times unrelated to injections were excluded from microscopic study because they were partly eaten or their organs autolyzed.

At autopsy, no gross abnormalities of the unopened heart were seen in any of the mice. Microscopic examination revealed cardiac lesions in a large percentage of both treated and control mice (Table II) without

TABLE II  
*Incidence of Combinations of Different Types of Cardiac Lesions in Control and Treated Mice*

	Total no. of mice	Total with lesions		One type		Two types		Three types		Four types		Five types	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Treated	86	71	82.6	22	25.6	22	25.6	17	19.8	7	8.1	3	3.5
Control	39	28	71.8	12	30.8	10	25.6	2	5.1	3	7.7	1	2.6

All percentages given are of the total number of mice examined in each group.

statistically significant difference in frequency and severity between the two groups. These lesions consisted of accumulations of cells of varying size in the myocardium, valve leaflets, endocardium, pericardium, and about the coronary vessels. The cellular elements in all of these sites were similar, consisting predominantly of lymphocytes, plasma cells, large mononuclear cells, "Anitschkow myocytes," and rarely neutrophilic polymorphonuclear leukocytes and large cells with one or more nuclei and basophilic cytoplasm.

In the valve leaflets these accumulations varied from a few cells beneath the endocardium of the superior surface of the leaflet to large focal accumulations which enlarged and distorted the leaflet (Figs. 1, 2, 3). At times diffuse infiltration of the entire leaflet and its chordae tendineae was present. The mitral was the valve most frequently involved and occasionally there were multiple foci in the same leaflet or in two leaflets. In only rare instances were accumulations seen in the aortic or tricuspid leaflets.

Small collections of the described cells in the myocardium (Fig. 4), with some tendency toward perivascular localization and a distinct predilection for the base of the interventricular septum, comprised the



most frequent lesion observed in both the control and treated mouse hearts. Fatty and hydropic degeneration and fragmentation of muscle fibers were frequently present with the cellular infiltration. Focal accumulations of the same cellular elements were seen in the pericardium and endocardium (Fig. 5) and, when present, most commonly involved the right atrium. Perivascular infiltration of a minor degree was common about the small and medium-sized coronary arteries (Fig. 6) and consisted of cells similar to those in the other lesions. In some instances the infiltrations were more dense, and in these an occasional cell resembling an Aschoff cell was present. In no instance, however, was a true "Aschoff nodule" observed nor was there collagen degeneration of the coronary vessels or perivascular tissues. An acute segmental fibrinoid degeneration of the aorta and main pulmonary artery with infiltration of polymorphonuclear leukocytes in the wall and surrounding tissue, resembling human periarteritis nodosa, was encountered in a very few instances (Fig. 7).

"Valvulitis" and perivascular infiltrations are considered here as the more significant from the standpoint of "rheumatic-like" lesions. The incidence of these lesions and of the other types described is given in Table III. In no instance is the difference in incidence between the treated and control groups of significance when the results are subjected to statistical analysis ( $X^2$  test).

TABLE III  
*Incidence of the Different Types of Lesions in Treated and Control Mice*

	Total no. of mice	Valvulitis		Perivascular infiltration		Myocarditis		Endocarditis		Pericarditis	
		No.	%	No.	%	No.	%	No.	%	No.	%
Treated	86	33	38.4	36	41.9	59	68.6	13	15.1	15	17.4
Control	39	8	20.5	11	28.2	25	64.1	9	23.1	3	7.7

All percentages given are of the total number of mice examined in each group.

The total incidence of one or more lesions in each group and the frequency of occurrence of combinations of the different lesions are given in Table II. There is no statistically significant difference between the treated and control groups in the total number of mice showing one or more lesions or showing different combinations of these lesions. Each of the lesions occurred alone but none with any more frequency than the others. All possible combinations of the lesions occurred, and here again no one combination was present with more regularity than any other. It is apparent from the table that slightly more than half of the mice had one or two of the different types of lesions while in only a very few were all of them present.

The severity of the lesions was arbitrarily graded from 1 plus to 3 plus and the incidence of the three grades compared in the treated and control groups (Table IV). Here again, no statistically significant difference between the two groups was found.

TABLE IV  
*Severity of the Different Types of Lesions in Treated and Control Mice*

	Total no. of mice	Valvulitis			Perivascular infiltration			Myocarditis			Endocarditis			Pericarditis		
		+	++	+++	+	++	+++	+	++	+++	+	++	+++	+	++	+++
Treated	86	13.9	13.9	10.5	33.7	8.1	0	39.5	22.1	6.9	15.1	0	0	13.9	1.2	2.3
Control	39	0	12.8	7.7	15.4	10.3	2.6	33.3	23.1	7.7	15.3	5.1	2.3	5.2	0	2.6

Figures given are percentages of the total number of mice examined in each group.

It may be stated that among the treated mice there was no statistically significant difference in the incidence or severity of lesions between those treated by intravenous and intraperitoneal injections of egg white; there was no difference between the mice which received only four injections and those which received five to fourteen; moreover, there was no difference between those dying of anaphylactic shock and those which survived multiple injections and were killed at intervals of 1 week. In the control group, there was no difference in the incidence or severity of lesions between those which were given a single injection of egg white and those not given any injections. Analysis of all the mice on the basis of age revealed no difference among those which died or were killed at intervals of 5½ to 16½ weeks.

## DISCUSSION

The results indicate that the normal Swiss mice of a "typhoid free" strain used in these experiments frequently have spontaneous cardiac lesions which are prominent. Treatment of these mice with multiple parenteral injections of diluted egg white produced anaphylactic hypersensitivity but failed to increase the incidence or severity of these lesions and also failed to produce cardiac lesions of any other type. In spite of the infrequent presence of collagen degeneration, such other features as the distribution of the spontaneous lesions, their cellular elements, and the occasional occurrence of segmental arterial fibrinoid degeneration indicate that these lesions are similar to those experimentally produced in rabbits in association with anaphylactic hypersensitivity.

Moore and his co-workers,<sup>7</sup> using a "typhoid resistant" strain of mice raised in their laboratory, reported that all their mice given multiple parenteral injections of diluted egg white developed cardiac lesions of the rheumatic type. However, they noted lesions of the same type in their

control mice but these were less frequent and less severe than in the treated group. The combination of a greater dosage of foreign protein and a different strain of mice used by these workers may be responsible for the greater incidence and severity of the lesions reported by them in treated mice.

Lesions similar to those described here but more characteristic of Aschoff nodules have been described in mice by Lenke and Loewe,<sup>8</sup> who believed them to be due to a spontaneous disease in mice resembling the rheumatic process in humans. Moore and his associates<sup>7</sup> believed that the cardiac lesions seen in their mice were the result of hypersensitivity to some factor in the mouse environment. We offer no suggestion as to the nature of the lesion in our own stock mice. Nevertheless, because of the frequency and severity of the cardiac lesions in normal animals, whether due to a latent infection, hypersensitivity to some environmental factor, or other unknown causes, these mice are obviously unsuitable for experiments in which the occurrence of "rheumatic-like" lesions is a determining factor in the interpretation of the results.

#### SUMMARY AND CONCLUSIONS

A high percentage of Swiss mice used as controls were found to have cardiac lesions resembling those of the rheumatic type associated with experimental anaphylactic hypersensitivity.

Treatment of these mice with multiple parenteral injections of egg white, although it produced anaphylactic shock, failed to influence the incidence and severity of these lesions and did not produce other cardiac lesions.

The high incidence and the severity of the cardiac lesions in the control mice used in these experiments indicate that some mice are unsuitable for the experimental investigation of hypersensitivity as related to the production of "rheumatic-like" lesions.

The cause of the observed cardiac lesions was not determined.

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[ *Illustrations follow* ]

## DESCRIPTION OF PLATES

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### PLATE 189

- FIG. 1. Heart of control mouse, showing an accumulation of cells beneath the endothelium of the mitral valve with enlargement and distortion of the leaflet. Hematoxylin and eosin stain.  $\times 150$ .
- FIG. 2. Higher magnification of the leaflet seen in Figure 1, to show cellular elements. Hematoxylin and eosin stain.  $\times 600$ .
- FIG. 3. Mitral valve of another control heart to show focal accumulation of cells. Hematoxylin and eosin stain.  $\times 600$ .

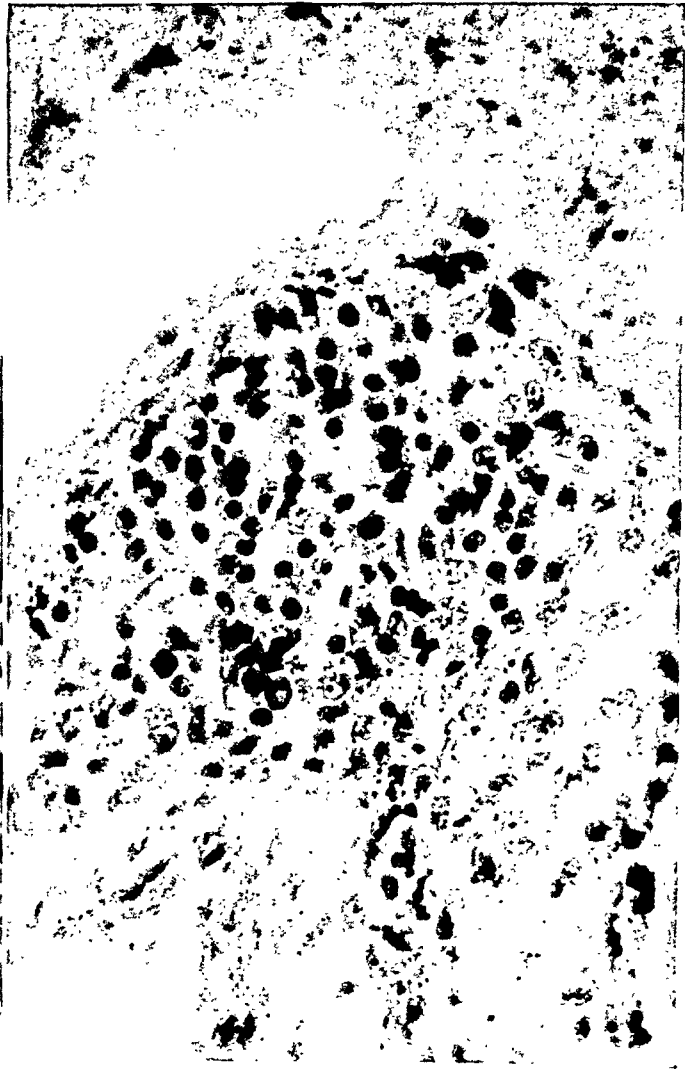
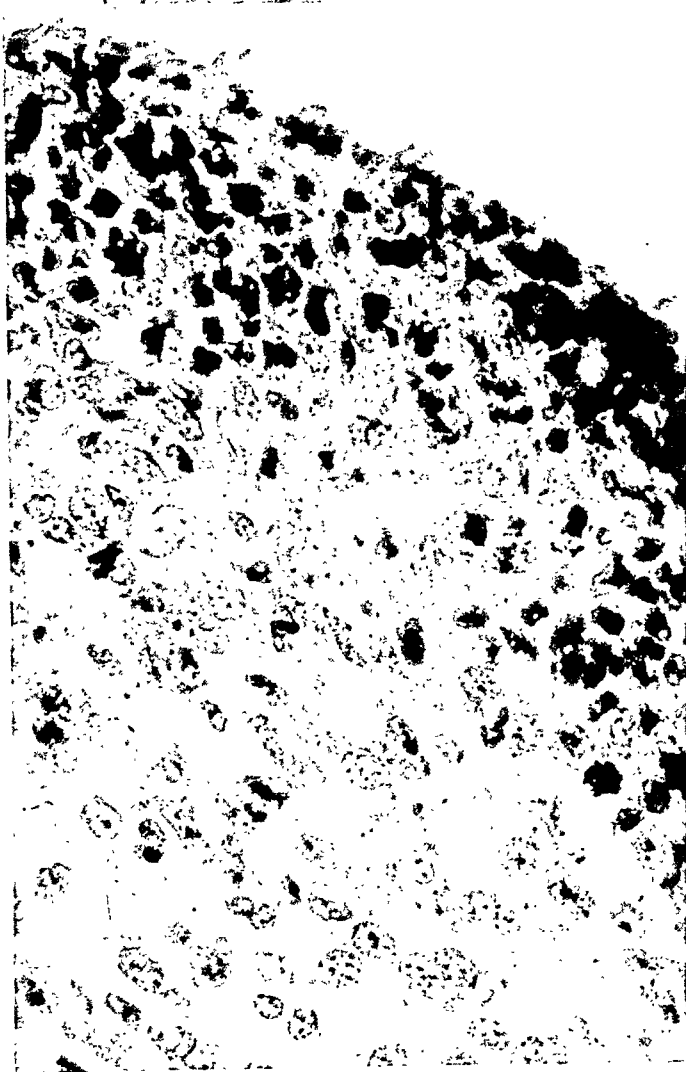
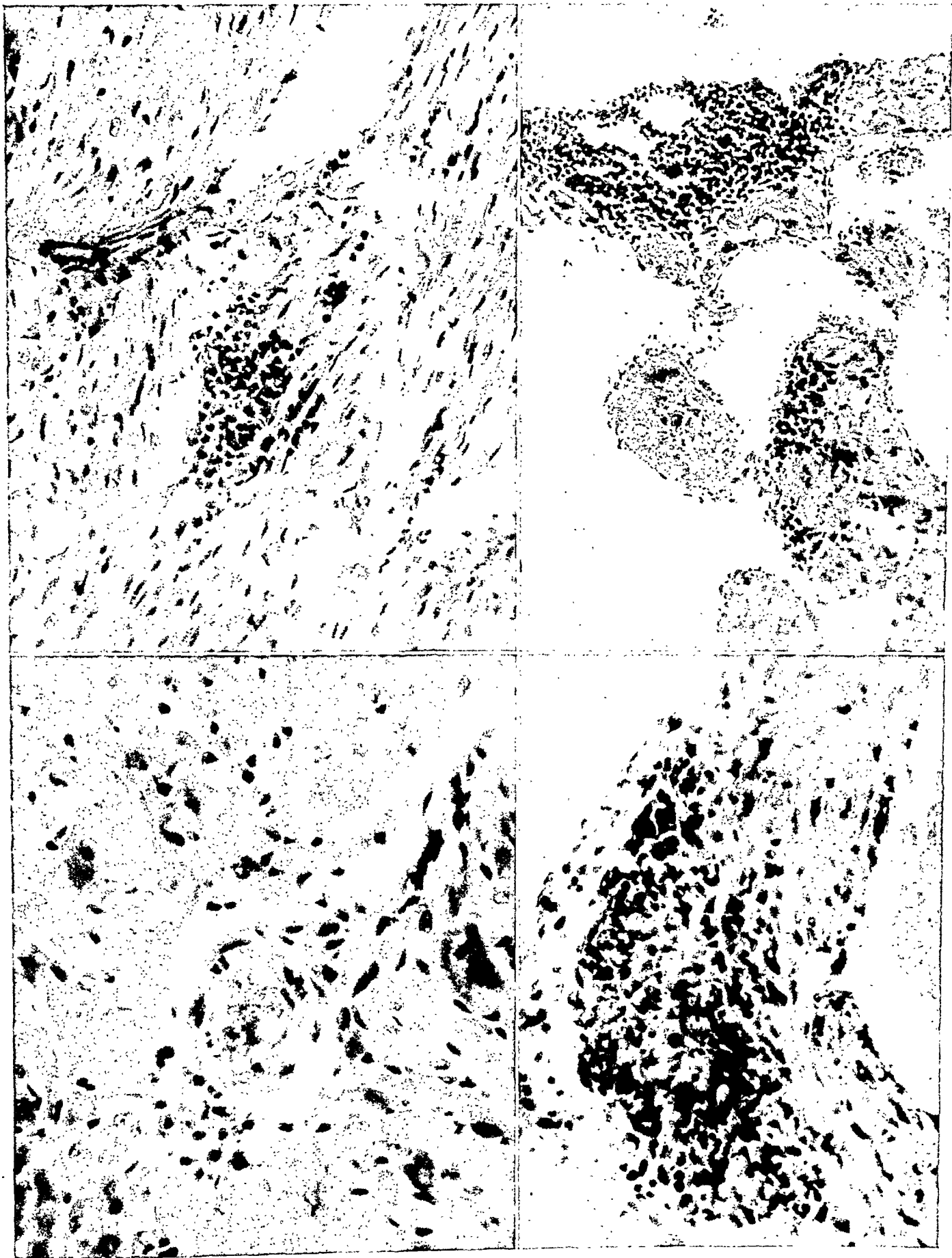


PLATE 190

- FIG. 4. Focal interstitial collection of cells in myocardium of heart of control mouse. Hematoxylin and eosin stain.  $\times 300$ .
- FIG. 5. Left atrium of a control heart showing cellular infiltration of pericardium and endocardium. Hematoxylin and eosin stain.  $\times 245$ .
- FIG. 6. Slight infiltration about a coronary artery in a control heart. Hematoxylin and eosin stain.  $\times 510$ .
- FIG. 7. Ascending aorta of a control mouse showing segmental degeneration and cellular infiltration. Hematoxylin and eosin stain.  $\times 360$ .

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Gray

Spontaneous Cardiac Lesions in Mice





## CHRONIC PANCREATITIS AND LITHIASIS

### I. A CLINICOPATHOLOGIC STUDY OF 62 CASES OF CHRONIC PANCREATITIS\*

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The relationship of chronic pancreatitis to antecedent acute attacks of the disease and to complications such as lithiasis, diabetes mellitus, and steatorrhea is gradually undergoing clarification. Pathogenesis and treatment are still major problems. The more complete elucidations of the clinical course and natural history of the primary disease of the pancreas uncomplicated by any disease of contiguous organs has recently been given by Comfort, Gambill, and Baggenstoss,<sup>1</sup> and by Maimon, Kirsner, and Palmer.<sup>2</sup> These workers have used the terms "chronic relapsing" and "chronic recurrent" pancreatitis to indicate the repetitive nature of the injury to the organ, presumably by acute episodes of one form or another.

In approaching the problem from the standpoint of pathology and attempting to correlate such clinical and physiologic data as seem applicable, we have reviewed the records of 33,500 consecutive autopsies at the Los Angeles County Hospital from August 31, 1926, to March 1, 1947. All instances of moderate to severe chronic diffuse pancreatitis and/or calculi have been used for this study. Excluded are those in which pancreatitis was secondary to perforating peptic ulcer or to obstruction of the ducts by carcinoma. Those with interacinar fibrosis alone, atrophy, and diabetes were not used. Particular attention has been paid to the genesis of calculi, and for that reason part II of this series† will be devoted entirely to that subject.

#### HISTORICAL LITERATURE

A complete review of the literature of chronic pancreatitis will not be attempted. Chronic pancreatitis was a more popular subject for authors before the time of Opie's contribution than it has been in recent decades. Opie<sup>3</sup> devoted a chapter numbering 39 pages to its discussion in 1910. Authors of recent textbooks of pathology have given a surprisingly small amount of space to it, or have failed to mention it altogether. The development of the present concept of chronic pancreatitis can be traced fairly well in the works of Claessen<sup>4</sup> in 1842, Friedreich<sup>5</sup> in 1878,

\* Received for publication, October 11, 1948.

† Will appear in the January issue of this *Journal*.

Oser<sup>6</sup> in 1898, Opie in 1902<sup>7</sup> and 1910,<sup>3</sup> Mayo Robson and Cammidge<sup>8</sup> in 1907, Gruber<sup>9</sup> in 1929, and in the recent publications of Comfort *et al.*<sup>1</sup>

No one seems to have been given credit for the first valid description of the disease. Claessen,<sup>4</sup> in 1842, in his monograph on diseases of the pancreas stated that much confusion had existed prior to that time in regard to various states of sclerosis and hardening of the pancreas. He clearly defined chronic inflammation of the pancreas and gave criteria distinguishing it from carcinoma. He reported one case and reviewed the previously reported histories of eight others which he considered to be representative of the same disease. Claessen mentioned that acute inflammation may be present in the pancreas of patients with chronic pancreatitis at autopsy and specifically stated that acute attacks may lead to the chronic disease. He illustrated this with a case report from the literature. Probably Claessen was the first author to emphasize and define the entity of chronic pancreatitis.

Friedreich<sup>5</sup> differentiated between primary and secondary chronic interstitial pancreatitis. The primary form he considered extremely rare. He wrote: "The most frequent form of chronic pancreatitis is the chronic interstitial inflammation, consisting of a hyperplasia of its interacinous connective tissue and consequent atrophy, even to the entire disappearance, of the gland-substance proper."<sup>5a</sup> Closure of small ducts leads to the formation of cysts. Interestingly enough, he recognized the rôle of alcoholism, as did many other early authors. He stated: "I am inclined to believe that a general, chronic, interstitial pancreatitis may result from excessive alcoholism (drunkards' pancreas)."<sup>5b</sup> He gave a case report with autopsy to demonstrate this relationship. Also of interest in view of our present-day concept is his mention of Klob's<sup>10</sup> report (1860) of chalky areas and hemorrhage in the thickened connective tissue of the pancreas. This probably represents the first reported case of chronic pancreatitis with death in a recurrent acute attack. The nature of fat necrosis, of course, was not recognized until Balser's<sup>11</sup> publication in 1882.

Friedreich<sup>5</sup> listed the symptoms of steatorrhea, diabetes, chronic jaundice, epigastric pain, and weight loss much as we describe them today. No mention was made of the possibility of calculi being secondary to chronic pancreatitis, but always vice versa.

Oser<sup>6</sup> recognized two types of pancreatitis. The first involved the entire gland and might be due to injurious agents entering along various avenues such as vessels, ducts, and lymphatics. Biliary disease and

alcoholism were considered in the etiology. He mentioned that atrophy and fibrosis followed stasis. A second group due to penetrating lesions of the stomach and duodenum he described as circumscribed pancreatitis. Opie<sup>7</sup> distinguished two forms of the disease: chronic interlobular and chronic interacinar pancreatitis. Chronic interlobular pancreatitis is the more common, usually secondary to duct obstruction, and often leads to great atrophy of the organ. However, the islets are resistant to destruction and diabetes is not seen as often as in the chronic interacinar type, which produces fibrosis in the centers of the lobules. This type of disease he found more frequently associated with chronic alcoholism and cirrhosis than the interlobular form.

Quénu and Duval,<sup>12</sup> at the time Opie was studying the pancreas, emphasized that stones in the common duct, if there for years, would cause chronic pancreatitis.

Chronic alcoholism as a cause of chronic pancreatitis was discussed by Lefas,<sup>13</sup> Lando,<sup>14</sup> and Poggenpohl.<sup>15</sup> Poggenpohl further refined the descriptions of fibrotic changes in the pancreas to include periacinar and intra-acinar forms.

After Opie there is a paucity of reports of any combined clinical and pathologic studies. Most reports were of a clinical nature only, or simply case reports. Several authors postulated that chronic pancreatitis was a complication of gallbladder disease in the absence of stones in the common duct. Gruber<sup>9</sup> reviewed the literature and wrote a complete description of the changes in the pancreas. More recently, French workers<sup>16,17</sup> have described a left chronic pancreatitis characterized by pain and tenderness in the left upper quadrant with acute attacks of variable severity which tended to recur for years. The pain radiated to the left and might be confused with gastric ulcer. These authors, along with others,<sup>18</sup> believed that repeated attacks of edematous pancreatitis gave rise to chronic pancreatitis. That it so often affected only the left half of the pancreas they attributed to the fact that the head of the organ has a dual blood supply and drainage system. If either the duct of Wirsung or the duct of Santorini was blocked, the other would carry the external secretion to the duodenum. They pointed out that the body and tail of the pancreas, however, have only one blood supply and one duct. Thus they are more susceptible to damage by blockage of the duct of Wirsung or to any interference with the vascular supply. For this disease they performed a number of left hemipancreatectomies.

Many of the French authors,<sup>19</sup> as well as others, emphasized that chronic disease may involve principally the head of the pancreas, pro-

ducing icterus, and may be difficult to distinguish from carcinoma. Peterson and Cole<sup>20</sup> believed that there are two types of involvement of the head causing jaundice. One is a localized pancreatitis which may subside in a few months. The other is due to diffuse sclerosis of the gland and produces an irreversible obstruction or stricture of the common duct.

That repeated attacks of pancreatitis might be associated with diabetes and steatorrhea was suggested by Stern<sup>21</sup> in 1930 and Svartz<sup>22</sup> in 1931. Each reported a single case. During the acute episodes the diabetes became much worse. Although Svartz used the term "recidivating pancreatitis," the cases reported by both authors probably represent the entity now known as chronic relapsing or chronic recurrent pancreatitis. One would like to have seen higher diastase levels in both patients at the time of the acute attacks in order to make the diagnosis unequivocal.

In 1946 Comfort *et al.*<sup>1</sup> reported a group of 29 patients with chronic relapsing pancreatitis in whom there was no complicating disease of contiguous organs. They carefully traced the natural history of the disease, emphasizing its recurrent nature and the sequelae of steatorrhea, diabetes mellitus, and calculi. The characteristic laboratory findings of various stages of the disease were brought to light. Alcoholism was present in 68 per cent of those in whom a history of alcohol consumption was elicited. There were 3 autopsies in this group. The authors considered interstitial fibrosis and residual necrosis of tissue as the most impressive findings, probably caused by repeated attacks of edematous pancreatitis or sublethal attacks of acute hemorrhagic pancreatitis with necrosis. Pseudocysts, perineural distribution of lymphocytes, and arteriosclerosis were described also. They were the first to emphasize that calculi are often secondary to pancreatitis.

Maimon *et al.*<sup>2</sup> observed a group of 20 patients with the same disease and termed it "chronic recurrent pancreatitis." They discussed the symptoms, laboratory diagnosis, and treatment. One autopsy was performed but no details were given. Only 2 of the group were alcoholic.

It should be remembered that these recent investigations have delineated a clinical entity not difficult to diagnose. Whatever the clinical course, symptomless or not, the resultant pathologic changes in the pancreas constitute the same disease that has been known for over 100 years. Although the terms "recidivating," "relapsing," and "recurrent" are excellent for clinical diagnosis, the question of the wisdom of using a multiplicity of terms for the same disease arises. So, for the pathologist at least, the continued use of the simple term "chronic pancreatitis" for anatomic diagnosis seems advisable.

## CLINICAL FEATURES

After microscopic examination of the pancreas we discarded all cases with minimal lesions. There remained 62 instances of chronic pancreatitis among 33,500 necropsies (0.18 per cent). After a review of the clinical records an attempt was made to evaluate the problem of diagnosis and the relation of pain, alcoholism, diabetes, and jaundice to the disease. The clinical diagnoses were disappointing. Not a single case was suspected or diagnosed as having chronic pancreatitis. The explanation for this probably lies in (1) inadequate histories, (2) lack of pain as an outstanding symptom, and (3) a low "index of suspicion" on the part of the attending and resident clinical staff, who only recently have become more aware of the entity.

Epigastric pain was certainly not a prominent complaint. It was mentioned in 16 (25 per cent) but could reasonably be attributed to coronary disease, pneumonia, cholelithiasis, cirrhosis, peritonitis, or pulmonary tuberculosis. However, in retrospect it is probable that the pancreas was the source of the pain in 4 cases. A diagnosis of acute pancreatitis was established by high serum amylase values in one of the latter. However, only 2 other patients in the entire series had a diagnostic serum amylase determination.

The correlation of chronic pancreatitis with alcoholism (24 instances) and with alcoholism plus fatty cirrhosis (19 cases) was most striking. In addition, 3 patients had portal cirrhosis without a history of alcoholism. Acute pancreatitis occurring during or following the ingestion of alcohol is well known.<sup>23-27</sup> Paxton and Payne,<sup>28</sup> in a report of 307 cases from this hospital, noted a history of excessive alcoholic intake in 55 (18 per cent). Therefore it is not surprising that a considerable number of chronic alcoholic patients with cirrhosis develop chronic pancreatitis. The minor episodes leading to a scarred pancreas could have been masked by alcoholism or diagnosed wrongly as acute gastritis. At the same time it is to be remembered that a majority of alcoholic patients dying of cirrhosis have a normal-appearing pancreas or present only minimal evidence of pancreatic disease.

Damage to the islet mechanism was considered sufficient to cause diabetes mellitus in 18 (29 per cent), a figure which probably would have been higher if glucose tolerance tests had been done. Sixteen of these had diabetes alone, while only 2 had a history of alcoholism complicated by cirrhosis. The total of 18 seems rather high for a group in which not a single instance of steatorrhea was noted. This may be explained by the lack of routine examination of the stools for fat and also by the fact that if diabetes mellitus is diagnosed ante mortem, attention

is called to the pancreas, which is then usually examined post mortem with care. Further, if a patient with chronic pancreatitis and undiagnosed steatorrhea dies of some other condition, the pancreas may be subjected to only a cursory inspection and the lesion be missed.

Jaundice due to chronic pancreatitis has been a popular subject in medical literature. Claessen<sup>4</sup> mentioned a case in an alcoholic patient. It was noted in 3 patients of our study: 2 of these died following operation for the relief of jaundice and the third of a liver abscess. In the third the obstruction was chronic enough to cause biliary cirrhosis. None of the 3 cases was associated with gallstones. In the fourth instance the gallbladder contained stones but its wall was thin and no adhesions or serosal changes were noted. At operation the surgeon thought the obstruction of the common duct was due to fibrosis of the head of the pancreas. This fourth patient we observed on the ward in an attack of acute pancreatitis. At necropsy no stones were seen in the common duct. We consider this another instance of icterus due to chronic pancreatitis.

Other clinical features included an equal distribution in respect to sex, 31 females and 31 males. This is actually weighted in favor of the females because 61 per cent of all autopsies in our hospital are on males. The average age for males and females was the same, 53 years; the oldest patient was 83 and the youngest 25.

#### GROSS PATHOLOGIC FINDINGS

The gross appearance of the pancreas at necropsy is characteristic in severe forms of the disease, especially when perilobular fibrosis dominates. The surface is irregularly nodular. This is seen best in the lesser peritoneal cavity. Adhesions occur here frequently. Increased resistance to cutting depends on the degree of fibrosis and may call attention to the disease. Dilatation of the ducts is common and a routine dissection of the duct of Wirsung is mandatory. The duct of Santorini may be explored with a probe after the duct of Wirsung has been opened. We have seen the dilated ducts filled with thick gray fluid, with a consistency approaching that of jelly. No evidence of stricture blocking the ducts was noted in this series.

In diffuse fibrosis the gross appearance of the gland may be deceptive and diagnosis may depend on microscopic examination. Reduction in the size of the pancreas may or may not occur. This depends upon whether atrophy or fibrosis and inflammation predominate. The gland may even be above normal weight.

A variety of complications were observed in the 62 cases studied. Pseudocysts were noted in 6. Terminal acute pancreatic necrosis was

present in 16 and in 4 it was the cause of death; in the remainder it varied from a few foci to many areas of recent necrosis and hemorrhage. Some were healing and one had gone on to abscess formation. Other complications in this series included one instance of obstruction of the splenic vein and 3 of obstruction of the common duct. Biliary cirrhosis was present in 2 of the latter. We included in this study all cases with stones in the ampulla of Vater causing chronic pancreatitis, in order to evaluate the incidence of this etiologic factor. This combination was diagnosed only 4 times in the 33,500 autopsies. In 2 of these no sections of the pancreas were available, so only 2 are included in the 62 cases. Later, in a study of acute pancreatitis, we will show the low incidence of stone in the ampulla as a cause of fatal pancreatitis. Twelve (20 per cent) of this group had stones in the gallbladder without evidence of disease of the common duct. This incidence is somewhat higher than the 13 per cent observed in adults in the entire autopsy series.

#### MICROSCOPIC PATHOLOGIC FINDINGS

*Normal and Controls.* A better understanding of the changes in chronic pancreatitis is obtained if certain details of the normal histologic structure are recalled. Although there is considerable variability, primary lobules as such rarely exceed 2.5 mm. in diameter. The majority are 1 mm. or less. Often the margins are ill defined and groups of lobules form secondary lobules up to 5 or 6 mm. in diameter. These larger masses we have noted especially near the axial center of the pancreas, but these also give the pancreas its external appearance. Occasionally, even a primary lobule seems to have a distinct separation into smaller portions. The connective tissue between the primary lobules is scanty. It may be a little more abundant between secondary lobules.

The blood supply is peculiar in one respect; in general, the vessels are dissociated from the duct system, entering the septa between lobules while the ducts enter the lobules and tend to be enveloped by them. Only rarely does one see a duct and venule or arteriole side by side. This factor is of more importance in acute pancreatitis, where thrombosis is common.

Control material was taken from individuals of all decades. In older people there may be some increase in perilobular connective tissue, fatty infiltration between the lobules, ductal epithelial hyperplasia, and atrophy. The last is of particular interest because it may be focal in character, involving one or two lobules only. In such areas, also, there may be hyperplasia of the epithelium of the ducts. These changes are similar in some respects to those in chronic pancreatitis. In some instances they accompanied arteriosclerosis of severe degree, of which



they could be considered the result. In others no etiologic factor was noted. Bosanquet<sup>20</sup> found some degree of pancreatic fibrosis in 10 per cent of patients over 40 years of age. Even in the aged the pancreas may be indistinguishable from that of a young adult.

*Chronic Pancreatitis.* Because so many of the pancreases in this group were the seat of recent acute changes, some of which were in the healing stage, an unusual opportunity to study the histogenesis of chronic pancreatitis presented itself. Although most of these lesions have been recorded in the past, we will outline them and discuss particularly the rôle of lobular necrosis. The examination of histologic preparations with a hand lens may give the pathologist the best evidence of the degree of fibrosis and lobular atrophy. The most striking changes occurred in the connective tissue. Most recent authors use the descriptive terms "interlobular" and "intralobular" or "interacinar"; these are so similar that we will replace the first with the older term "perilobular." The proliferation of connective tissue was, in every instance except one, greater in the perilobular than in the lobular compartment. In 3 cases the fibrosis was best described as diffuse and no accentuation of lobular pattern could be discerned. The degree of both perilobular and intralobular fibrosis was graded from 1 plus to 4 plus. This grading was arbitrary and often based on one histologic section in the file, but it was nevertheless an approximate indication of the incidence of the various grades of fibrosis. The perilobular increase was usually one or two grades more severe than the intralobular fibrosis. Because the degree of fibrosis might vary from one part of the pancreas to another, we combined those with 1 plus and 2 plus perilobular fibrosis into one group and those with 3 plus and 4 plus change into a second group. Those with diffuse fibrosis form a third group. Table I presents the grading and the relationship to alcoholism, cirrhosis, and diabetes mellitus.

TABLE I  
*Relationship Between Perilobular Pancreatic Fibrosis and the Presence of Alcoholism, Cirrhosis, and Diabetes*

Perilobular fibrosis		Alcoholism	Cirrhosis	Diabetes
Grade	Number			
1+ and 2+	31	10	8	13
3+ and 4+	28	11	12	4
Diffuse	3	3	2	1
Total	62	24	22	18

The cellularity and maturation of the connective tissue varied widely with the age of the lesion. The older were densely fibrotic and relatively acellular. In the younger, connective tissue with more fibroblasts was

noted and the amount of collagen was not so striking. The perilobular connective tissue surrounded lobules of variable size; in some instances it seemed that larger lobules had been broken up into several smaller ones. Thus the distinction between perilobular and intralobular connective tissue becomes a matter of interpretation. In the 3 pancreases in which fibrosis was diffuse, no difference in severity between the intralobular and perilobular areas was noted. In 2 of these there was some tendency toward obliteration of the lobular pattern. In the third no evidence of lobules could be seen in most of the tissue (Fig. 1). The diffuse fibrosis had separated acini and surrounded islets and ducts indiscriminately. This occurred in a 52-year-old Caucasian who admitted drinking one-fifth of a gallon of whiskey daily for many years. He died of Laennec's cirrhosis. We have seen no report describing diffuse fibrosis of the pancreas of this type.

One important histologic finding related to the genesis of the disease should be emphasized. This is destruction of portions of lobules or of entire lobules during an acute attack (Fig. 2). Usually this occurred in foci involving only one or two lobules. Such lobular destruction was present, however, throughout many sections of the pancreas. The necrotic acini were often in the periphery of the lobule. Various stages of connective tissue replacement of such lobules were seen. If only the peripheral rim of acini was destroyed, the new connective tissue would simply widen the perilobular bands. Finally a large proportion of the original lobule might be replaced by connective tissue (Fig. 3). A few acini, an occasional duct, and perhaps an islet would be all that remained. Where several lobules had been destroyed (Fig. 4), larger areas of fibrous tissue occurred (Fig. 5). Connective tissue replacement might subdivide some lobules into smaller ones with great distortion of the normal pattern. Infiltration of polymorphonuclear leukocytes, hemorrhage in the acute stage, and sooner or later many lymphocytes, completed the inflammatory picture. Often hemosiderin was the telltale sign of previous attacks of acute hemorrhagic pancreatitis.

It seemed that the end result in the pancreas depended on two factors: first, the proliferation of perilobular and intralobular connective tissue, resulting in the formation of lobules of variable size, and second, lobular destruction. Whether the first could follow minimal attacks of pancreatitis without necrosis could not be proved from histologic study. Changes in the remaining acini were few. Usually dilatation was not seen. As Rich and Duff<sup>30</sup> pointed out, the cells spread apart easily if there was increased back pressure, allowing the escape of fluid.

Alterations in the duct system included dilatation, hyperplasia, and

squamous metaplasia. The degree of dilatation of the ducts was variable, in general being in proportion to the severity of the fibrosis. As noted under gross pathologic findings, explanation of the dilatation is not easy, as mechanical obstruction was rarely described. The possibility of its being due to chronic inflammation and contraction of fibrous tissue, as in bronchiectasis, has to be considered. The hyperplasia of the ducts included both increase in numbers and an unusual epithelial hyperplasia (Figs. 6 and 7). As pointed out by previous authors,<sup>31</sup> there is the possibility that the numerical increase follows lobular destruction in much the same manner that bile duct hyperplasia follows destruction of functioning elements. That the numerical and epithelial hyperplasia might lead to neoplasia is logical. Yet we have not been able to prove this relationship in any pancreas. Many carcinomatous pancreases have chronic pancreatitis distal to the obstructing carcinoma, but this does not indicate that pancreatitis came first. A few lesions designated as chronic pancreatitis in this autopsy series, when reviewed, proved to be carcinomas.

The dilatation and hyperplasia of ducts often involved the smaller radicles, including the intercalary group (Fig. 8). This, coupled with the pink-staining debris within them, we believe, is important in the formation of calculi. It is difficult to explain the failure of the ducts to empty. Possible functional or organic failure of the acini to secrete enough juice to wash out the debris may be a factor.

Vascular sclerosis (Fig. 9) usually was most severe in areas of fibrosis and destruction; the action of trypsin in destroying vessels, as noted by Rich and Duff,<sup>30</sup> might have led to many of the chronic changes.

The degree of destruction of islets was difficult to evaluate because there was no way of knowing from what part of the pancreas the sections had been taken. The islets in 13 of the 62 cases showed some degree of destruction or degenerative change (Fig. 10). Seven of these patients had diabetes mellitus clinically. As is seen in Table I, the most severe degrees of pancreatitis (3 plus and 4 plus) were not so often associated with diabetes mellitus, while two-fifths of those with 1 plus to 2 plus change had diabetes. The most severe type of pancreatitis (4 plus) was often seen in alcoholic patients and the correlation of alcoholism and diabetes is not very close, as only 2 of the 24 alcoholic patients had diabetes. At least, one can say that the alcoholic person may develop severe chronic pancreatitis without diabetes mellitus. As mentioned in an earlier paper,<sup>32</sup> the destruction of islets may proceed at a rate parallel with that of the acini, and diabetes may thus supervene before steator-

rhea. The destruction of islets may result from their incorporation in areas of lobular necrosis or from fibrosis and ischemia. One difficulty in interpretation of the islet changes is the fact that we do not know how much actual destruction is necessary in any particular person before the diabetic state is established. It seems likely that some unknown mechanism is responsible in diabetic patients who have what appears to be sufficient islet tissue at necropsy.

The genesis of pseudocysts could be seen especially well in one case. In areas of necrosis and hemorrhage the liquefaction of tissue had formed many cyst-like spaces lined with shaggy sheets of necrotic material. In many instances necrosis had involved the parenchyma, and in one portion of the circumference of a cyst, ducts could be seen which apparently opened into it. Older cysts were present also, with recent areas of necrosis and hemorrhage in their walls, indicating activity of enzymes in a cyst with an acute exacerbation. Finally, pseudocysts may remain for years. Their thick fibrotic walls may even calcify.

One motivating purpose back of this study was the possibility of finding all stages of calculus formation and/or calcification of the pancreas in such a large group of chronically diseased organs. In spite of a most careful search, we were able to demonstrate small calculi in only one duct (Fig. 11) and two microscopic areas of calcification in fibrous tissue. It was impossible to decide if the latter represented calcification in diseased ducts or acini, or perhaps calcification in the interstitial tissue. From our study in this series and in a large experience with acute pancreatitis, we believe it is most unusual for calcification to occur primarily in the interstitial connective tissue of the pancreas or to persist in areas of fat necrosis. The reasons for this will be discussed in detail in Part II.

## DISCUSSION

In the study of this material, certain clinical and pathogenetic phases of chronic pancreatitis are manifest. First is its incidence in a large general hospital. No comparable statistics were noted in the literature. Our finding of 62 examples in 33,500 autopsies, or 0.18 per cent, probably explains the low "index of suspicion" of the clinicians and the difficulty in diagnosis. The paucity of symptoms and findings of chronic pancreatitis may be partially due to lack of interrogation and routine use of such laboratory procedures as serum amylase and stool analysis for fat. In this group, selected because of

unquestionable evidence of pancreatic damage, we were surprised to find so few in whom there was a history of acute attacks of pancreatitis such as described by Comfort *et al.*<sup>1</sup> Abdominal symptoms that might be related to the pancreas were noted in only 4 cases. However, the fact that acute pancreatitis was present at necropsy in 16 of the patients with chronic pancreatitis is strong evidence that the chronic changes were due to previous acute attacks.

The correlation noted in this series between the pancreatic lesion and alcoholism, with or without cirrhosis (24 cases), is similar to that found by others.<sup>23-27</sup> The occurrence of pancreatitis in alcoholic patients with fatty cirrhotic livers, we believe, has roughly paralleled the great increase of alcoholic cirrhosis noted in the Los Angeles County Hospital in the past 14 years. Evans and Gray,<sup>33</sup> in 1938, noted a rise from 0.65 to 1.84 per cent of all autopsies following the repeal of prohibition. From 1939 to 1946 it rose to 5.9 per cent of autopsies in subjects over 9 years of age.<sup>34</sup>

In this series the chronic alcoholic patients certainly had the highest incidence (10 of 24) of acute pancreatitis at necropsy. Once the pancreas has been the seat of an acute attack, the likelihood of further attacks seems enhanced, providing the same etiologic factors continue to operate. Paxton and Payne,<sup>28</sup> in their report on pancreatitis from this hospital, found that 61 per cent of their patients had a history indicating similar previous attacks. From studying the clinical course and seeing the lesions at necropsy one gets the idea that repeated episodes of subclinical character commonly occur. These are probably due to escape of pancreatic juice and would explain the stubborn chronicity noted clinically in relapsing pancreatitis. We have been impressed by the lack of evidence of pyogenic complications such as abscesses in both acute and chronic pancreatitis. All stages of the disease appear to be explainable by the enzymatic theory. Acute pancreatitis may be the terminal event also in a cirrhotic patient who is not consuming alcohol at the time. The mechanism of the production of pancreatitis by alcohol is not known but its relationship has been shown repeatedly.

That the fatty liver might be related to acute pancreatitis in some unknown way is suggested by the experiments of Groen.<sup>35</sup> He fed 9 dogs fat bacon exclusively. Death occurred, often suddenly, in 5 dogs within 5 to 7 months. Severe fatty infiltration of the liver was noted in all. Four of the 5 animals had acute pancreatic necrosis. Microscopically, the pancreatic ducts were blocked by an unidentified material which could have caused necrosis by obstruction.

The fundamental histologic change in chronic pancreatitis is fibrosis. This, in turn, is characterized by its great variability as to degree and results. It varies from one part of the pancreas to another and from perilobular to intralobular compartments. The quantity of acinar and islet atrophy is often unpredictable from a survey of the fibrous change. Duct changes likewise may not correspond to the degree of fibrosis. That fibrosis is due to one or more attacks of acute pancreatitis is certainly true in many instances, but in those patients without symptoms a definite etiologic factor cannot be assigned. Minimal or subclinical attacks of upper abdominal pain indicative of pancreatitis but forgotten by the patient are a possibility not to be dismissed.

The pattern of the fibrous change probably depends upon the severity of the acute attacks; edematous pancreatitis may result in fibrosis predominating in the perilobular spaces. In more severe attacks, as we have shown, lobular necrosis and replacement with fibrous tissue causes more extensive scarring and often a nodular pancreas. With repeated attacks, more and more acinar and islet tissue would be destroyed, finally resulting in a fibrosed, atrophic organ with deficiency in external and/or internal secretion. Strangely enough, in this series diabetes was the most common complication. Steatorrhea could have been overlooked because stools are not examined routinely for fat as is the urine for sugar.

One difficult problem that arose during this study was that of establishing histologic criteria to distinguish chronic pancreatitis, with extensive islet destruction and diabetes, from the atrophic gland containing hyalinized and fibrosed islets, seen frequently in elderly diabetic patients. Opie<sup>3</sup> described an interacinar form of chronic pancreatitis with destruction of islets in patients with diabetes mellitus. He considered that this was probably the same disease as that previously described as atrophy of the pancreas. It seems doubtful if such a change should be classified as a form of chronic pancreatitis. Although examples of this change were noted in the autopsy material, they were not included in the final study. Histologic evidence of true chronic pancreatitis includes dilatation of ducts and hyperplasia, round cell infiltration and lobular destruction with replacement fibrosis. Yet in healed pancreatitis of many years' duration an atrophic gland remains which on histologic examination alone presents a difficult problem in interpretation. This was shown by one of our cases, a white woman, 58 years old, who died in uremia due to pyelonephritis and nephrosclerosis in April, 1938. She had had a laparotomy for

acute pancreatitis in 1903. Diabetes mellitus was diagnosed in 1923. Upper abdominal pain and tenderness had been described on admittance to the hospital in 1934. At necropsy a large pseudocyst, 17 cm. in diameter, involved the body and tail of the pancreas. Its wall was calcified. Yet, on microscopic examination of the remaining pancreatic tissue, a diagnosis of chronic pancreatitis could be made only with difficulty. Only atrophy and fibrosis were seen. This case emphasizes the importance of the clinical story. Those patients in whom there is no gross or microscopic evidence of past acute episodes with resultant chronic changes should at least be put in a questionable diagnostic category.

The changes in the duct system are of interest. Dilatation may be severe, and when accompanied by sufficient destruction or functional failure of acini the ducts fail to empty and the inspissated pink-staining material seen microscopically accumulates in many of the branches. In this débris, calcium salts may be precipitated, as was noted once. Hyperplasia of ducts and their epithelium is probably reparative and the possibility of tumor formation exists.

Though chronic pancreatitis may become manifest by all the symptoms and signs that have been so well described, it is most puzzling that severe morphologic changes can occur in the organ and the patient remain symptomless.

Because of the incidence of acute pancreatitis, especially that associated with alcoholism, in such an institution as the Los Angeles County Hospital, it seems inevitable that chronic pancreatitis will in the future occupy a more significant position in clinical diagnosis than it has in the past.

#### SUMMARY AND CONCLUSIONS

Sixty-two instances (0.18 per cent) of chronic pancreatitis were found in 33,500 autopsies.

Twenty-four of the 62, or 38.7 per cent, gave a history of excessive use of alcohol. Nineteen of the alcoholic patients also had portal cirrhosis.

Eighteen, or 29 per cent, were diabetic. Diabetes was more often associated with moderate degrees of pancreatic fibrosis and only twice was it seen in alcoholic patients.

The difficulty in diagnosis of chronic pancreatitis is emphasized by the fact that none was recognized clinically. A history of abdominal pain typical of pancreatitis was elicited in only 4.

In contrast to the symptomless course, at necropsy 16 had evidence of acute pancreatitis; in 4 it was the cause of death.

The distribution of lobular necrosis is important in determining the pattern of the resulting fibrosis.

Perilobular fibrosis is the predominating change. Diffuse fibrosis of a peculiar type was noted once.

Early deposition of microcalculi in the ducts was seen once.

Biliary calculi impacted in the ampulla of Vater as a cause of chronic pancreatitis occurred twice.

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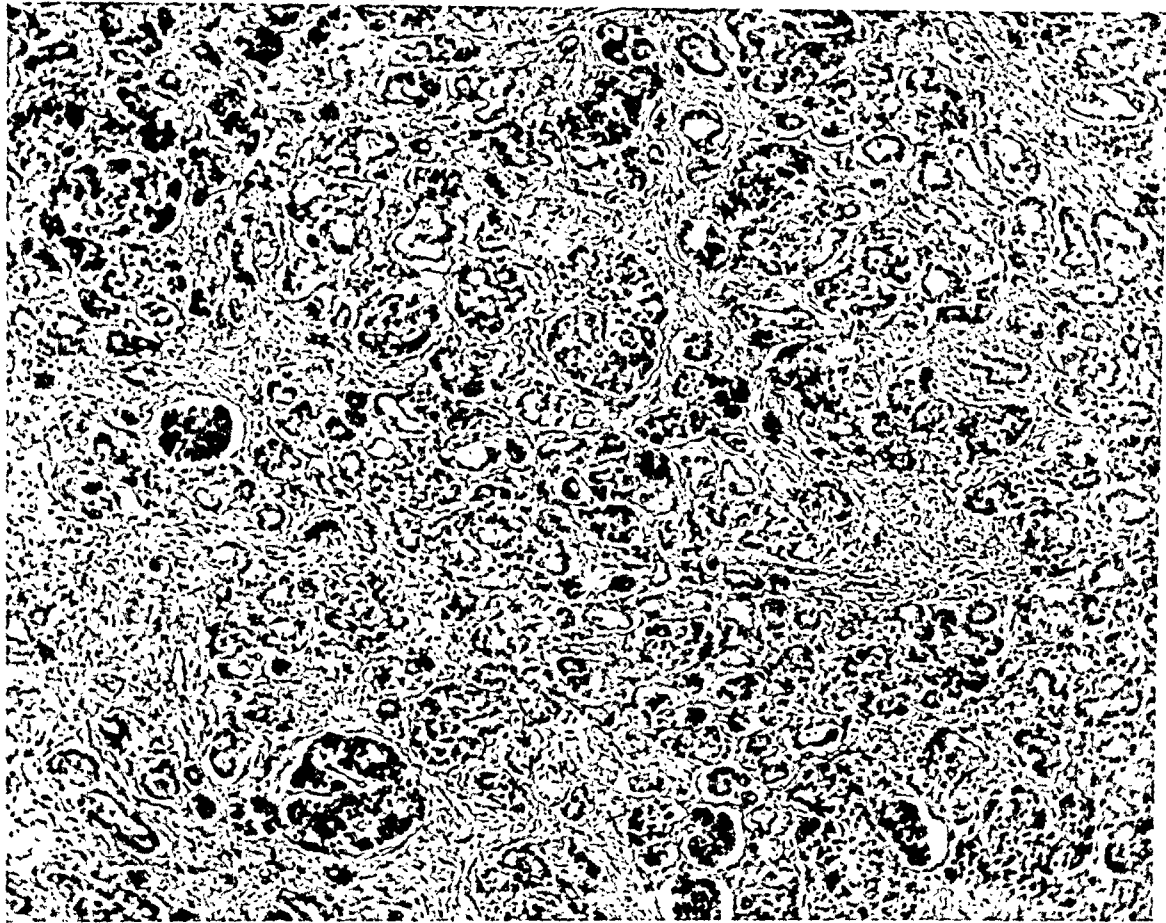
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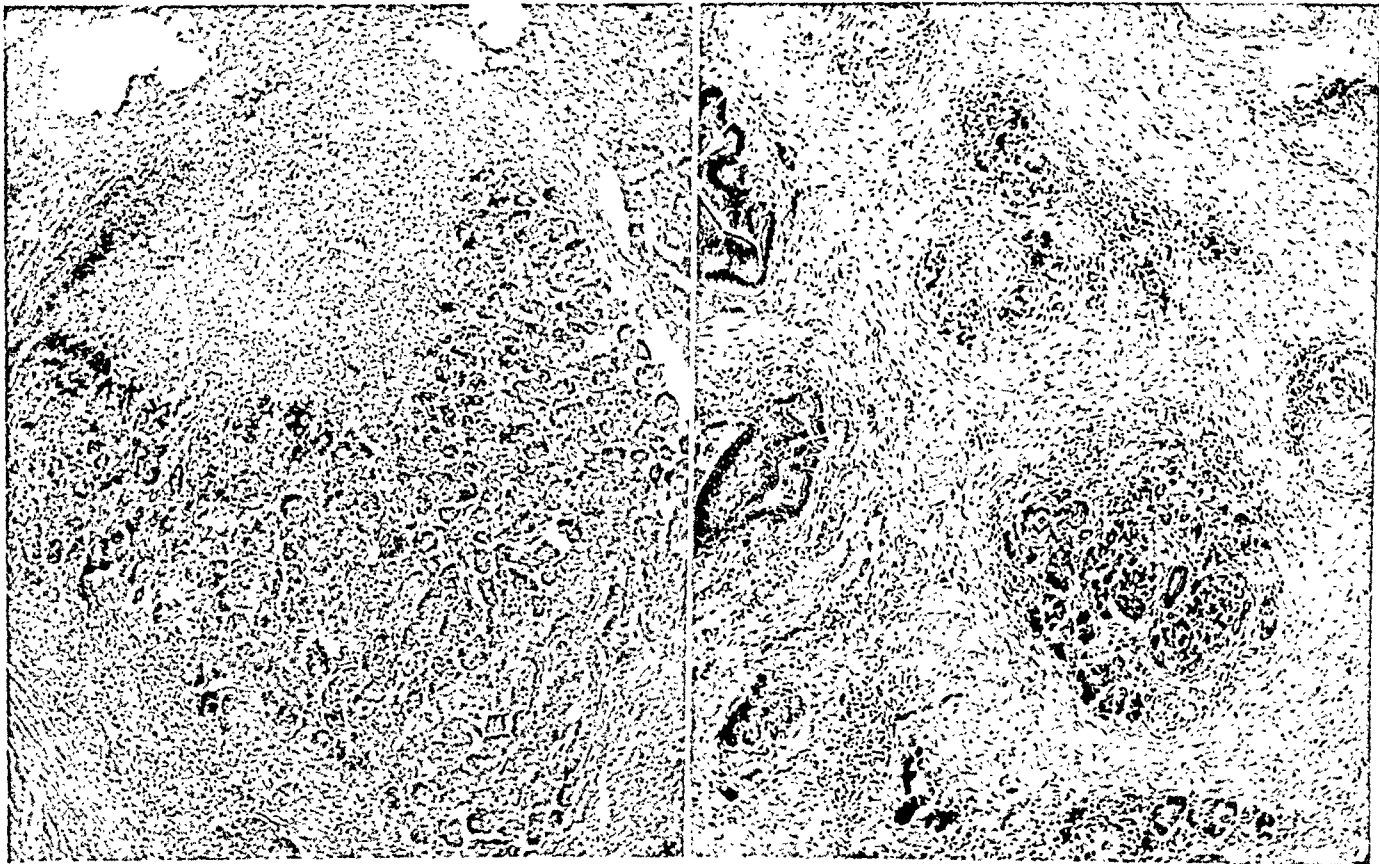
All illustrations were prepared from sections stained with hematoxylin and eosin.

#### PLATE 191

- FIG. 1. Diffuse fibrosis of the pancreas with obliteration of lobular architecture.  $\times 70$ .
- FIG. 2. Early necrosis of peripheral portion of a lobule. The perilobular fibrosis indicates chronicity.  $\times 63$ .
- FIG. 3. The connective tissue replacement of portions of lobules represents the end stage of partial lobular necrosis shown in Figure 2.  $\times 63$ .



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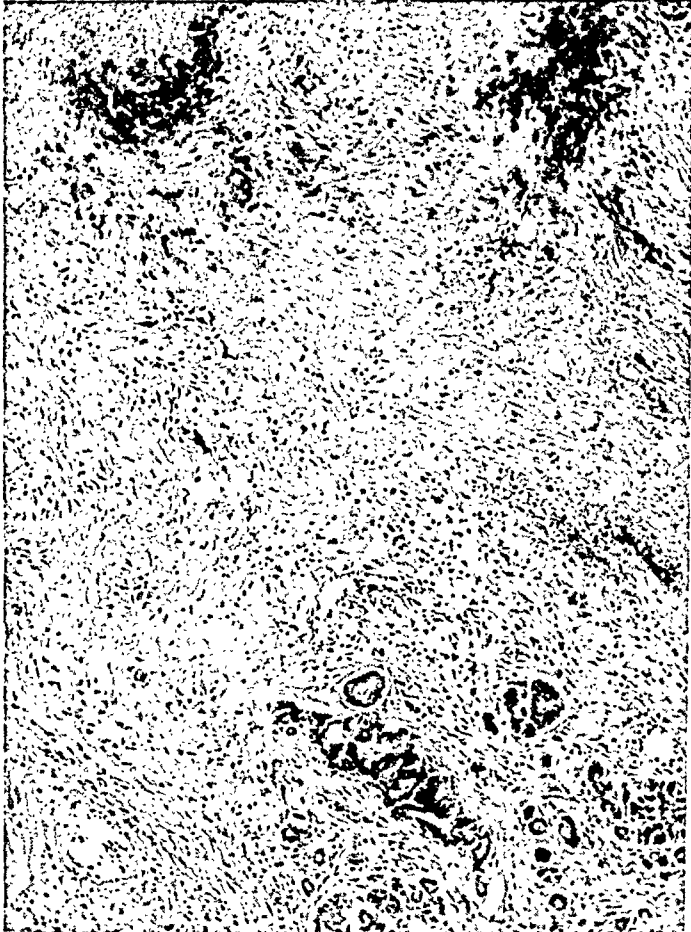
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Chronic Pancreatitis and Lithiasis

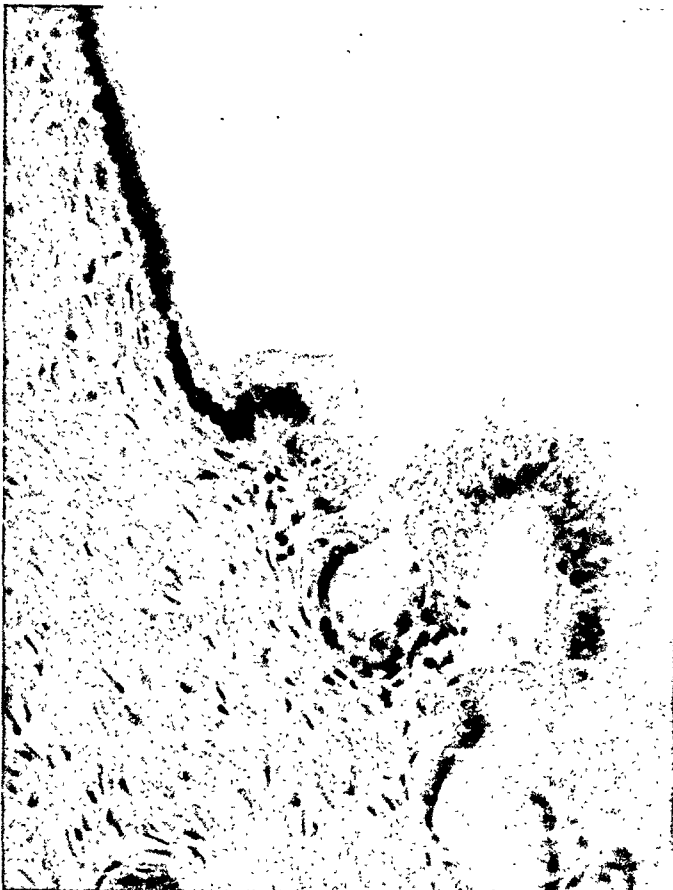
PLATE 192

- FIG. 4. Lobular necrosis involving several lobules. Connective tissue proliferation and round cells at margin of necrotic area indicate the beginning of healing.  $\times 68$ .
- FIG. 5. Extensive fibrosis following complete lobular necrosis, such as seen in Figure 4.  $\times 68$ .
- FIG. 6. Dilated duct showing a portion of its epithelium undergoing hyperplasia. Secondary duct lined with similar epithelium.  $\times 37$ .
- FIG. 7. Higher magnification of junction of normal and hyperplastic epithelium shown in Figure 6.  $\times 220$ .

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PLATE 193

FIG. 8. Dilatation and hyperplasia of ducts within a lobule.  $\times 132$ .

FIG. 9. Hyalinized remains of blood vessel in chronic pancreatitis.  $\times 70$ .

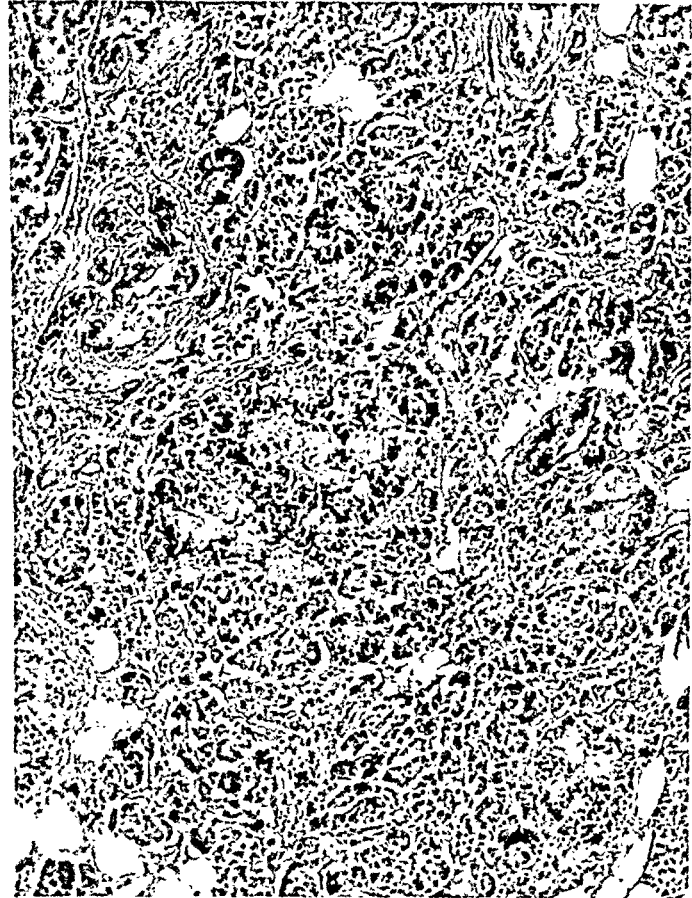
FIG. 10. Hyaline changes in islets of Langerhans. Chronic pancreatitis. History of chronic alcoholism. Diabetes mellitus not proved.  $\times 66$ .

FIG. 11. Small calculi precipitated in pink-staining débris of a dilated pancreatic duct.  $\times 79$ .

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